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Phthalates in the food chain

Measuring and modelling human exposure and impact on public health

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List of abbreviations

Chemical compounds

Abbreviation	Name (IUPAC)	Name (thesis)
3cx-MPP	2-[(3-Carboxypropoxy)carbonyl]benzoic acid	3Carboxy-mono-propyl phthalate
3-MCPD	3-Chloro-1,2-propanediol	3-Monochloropropane-1,2-diol
5cx-MEPP	2-[(2-Ethyl-6-hydroxy-6-oxohexoxy)carbonyl]benzoic acid	5Carboxy-mono(2-ethylhexyl) phthalate
5OH-MEHP	2-[(2-Ethyl-5-hydroxyhexoxy)carbonyl]benzoic acid	5OH-mono(2-ethylhexyl) phthalate
5oxo-MEHP	2-[(2-Ethyl-5-oxohexoxy)carbonyl]benzoic acid	5Oxo-mono(2-ethylhexyl) phthalate
ASE	Phenyl pentadecane-1-sulfonate	Alkylsulphonic phenylester
ATBC	Tributyl 2-acetoxy-1,2,3-propanetricarboxylate	Acetyl tri-*n*-butyl citrate
BBP	Benzylbutyl phthalate	Benzylbutyl phthalate
COMGHA	Mixture of 12-acetoxy-stearic acid, 2,3-bis(acetoxy)propyl ester and 2,3-diacetyloxypropyl octadecanoate	Mixture of 12-acetoxy-stearic acid, 2,3-bis(acetoxy)propyl ester and octadecanoic acid, 2,3-bis(acetoxy)propyl ester
DCHP	Dicyclohexyl phthalate	Dicyclohexyl phthalate
DEGD	Oxydi-2,1-ethanediyl dibenzoate	Diethylene glycol dibenzoate
DEHP	Bis(2-ethylhexyl)phthalate	Di(2-ethylhexyl) phthalate
DEHT	Bis(2-ethylhexyl)terephthalate	Di(2-ethylhexyl)terephthalate
DEP	Diethyl phthalate	Diethyl phthalate
DGD	Oxydi-3,1-propanediyl dibenzoate	Dipropylene glycol dibenzoate
DiBP	Diisobutyl phthalate	Diisobutyl phthalate
DiDP	Diisodecyl phthalate	Diisodecyl phthalate
DINA	Bis(7-methyloctyl) adipate	Diisononyl adipate
DINCH	Bis(7-methyloctyl) 1,2-cyclohexanedicarboxylate	Di-isononyl-cyclohexane-1,2-dicarboxylate
DiNP	Diisononyl phthalate	Diisononyl phthalate
DMP	Dimethyl phthalate	Dimethyl phthalate
DnBP	Dibutyl phthalate	Di-*n*-butyl phthalate
DnDP	Didecyl phthalate	Di-*n*-decyl phthalate
DnOP	Dioctyl phthalate	Di-*n*-octyl phthalate
DPHP	Bis(2-propylheptyl) phthalate	Di(2-propylheptyl) phthalate
DPP	Dipentyl phthalate	Di-*n*-pentyl phthalate
GTA	1,2,3-Propanetriyl triacetate	Glycerol triacetate
MBzP	2-[(Benzyloxy)carbonyl]benzoic acid	Mono-benzyl phthalate
MCHP	2-[(Cyclohexyloxy)carbonyl]benzoic acid	Mono-cyclohexyl phthalate

MCPP	2-[(3-Carboxypropoxy)carbonyl]benzoic acid	3Carboxy-mono-propyl phthalate
MECPP	2-[(2-Ethyl-6-hydroxy-6-oxohexoxy)carbonyl]benzoic acid	5Carboxy-mono(2-ethylhexyl) phthalate
MEHHP	2-[(2-Ethyl-5-hydroxyhexoxy)carbonyl]benzoic acid	5OH-mono(2-ethylhexyl) phthalate
MEHP	2-[(2-Ethylhexyloxy)carbonyl]benzoic acid	Mono(2-ethylhexyl) phthalate
MEOHP	2-[(2-Ethyl-5-oxohexoxy)carbonyl]benzoic acid	5Oxo-mono(2-ethylhexyl) phthalate
MEP	2-(Ethoxycarbonyl)benzoic acid	Mono-ethyl phthalate
MiBP	2-(Isobutoxycarbonyl)benzoic acid	Mono-isobutyl phthalate
MiNP	2-(Isononoxycarbonyl)benzoic acid	Mono-isononyl phthalate
MMP	2-(Methoxycarbonyl)benzoic acid	Mono-methyl phthalate
MnBP	2-(Butoxycarbonyl)benzoic acid	Mono-*n*-butyl phthalate
MnOP	2-[(Octyloxy)carbonyl]benzoic acid	Mono-*n*-octyl phthalate
OH-MiBP	2-[(2-Methyl-3-hydroxypropoxy)carbonyl]benzoic acid	3OH-mono-methylpropyl phthalate
OH-MnBP	3-[(Hydroxybutoxy)carbonyl]benzoic acid	3OH-mono-*n*-butylphthalate
TXIB	2,2,4-Trimethyl-1,3-pentanediyl bis(2-methylpropanoate)	Trimethyl pentanyl diisobutyrate

Other abbreviations

ADHD	Attention Deficit Hyperactivity Disorders
AGD	Anogenital distance
ALARA	As Low As Reasonably Achievable
BBN	Betabinomial-normal
BTF	Biotransfer factor
CMR	Carcinogenic, mutagenic or toxic for reproduction
CONSEXPO	CONSumer EXPOsure
Danish EPA	Danish Environmental Protection Agency
DNA	Deoxyribonucleic acid
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EDC	Endocrine disrupting compound
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic *Escherichia coli*
EN-forc	ENvironmental Food transfer model for ORganic Contaminants
EQS	Environmental quality standard
EUSES	European Union System for the Evaluation of Substances
FASFC	Federal Agency for the Safety of the Food Chain
FEV_1	Forced expiratory volume at 1 sec
FLEHS II	Second Flemish Environment and Health Study
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FSH	Follicle-stimulating hormone
FVC	Forced vital capacity
GC-EI-MS	Gas chromatography – low resolution – mass spectrometry with electron impact ionisation
GC-FID	Gas chromatography – flame ionisation detection
GPC	Gel permeation chromatography
HOMA-IR	Homeostasis model assessment – insulin resistance
LB	Lower bound
LH	Luteinising hormone
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of detection
LOQ	Limit of quantification
MAFF UK	British Ministry of Agriculture Fisheries and Food
MB	Medium bound
MC1	Measurement Campaign 1 of the PHTAL project
MC2	Measurement Campaign 2 of the PHTAL project
MCRA	Monte Carlo Risk Assessment
MRL	Maximum residue level
NCI	American National Cancer Institute
NOAEL	No Observed Adverse Effect Level

PAH	Polycyclic aromatic hydrocarbon
PBPK	Physiologically based pharmacokinetic
PBT	Persistent, bioaccumulative and toxic
PCB	Polychlorinated biphenyl
PEF	Peak expiratory flow
PET	Polyethylene terephthalate
PVC	Polyvinyl chloride
REACH	Registration, evaluation, authorisation and restriction of chemicals
RfD	Reference dose
RIVM	Dutch National Institute of Public Health and the Environment
RPM	Regional Population based Model
RSD	Relative standard deviation
SCCP	Scientific Committee on Consumer Products
SHBG	Sex-hormone binding globulin
SIM	Selected ion monitoring
SML	Specific migration limit
SPME	Solid phase microextraction
SVHC	Substances of very high concern
TDI	Tolerable daily intake
Tukey's HSD	Tukey's Honest Significant Difference
UB	Upper bound
US EPA	American Environmental Protection Agency
vPvB	Very persistent and very bioaccumulative
VITO	Flemish Institute for Technological Research
VLAREM	Flemish Environmental Permitting Regulation
VMM	Flemish Environment Agency
WFD	Water Framework Directive
WHO	World Health Organisation
XtraFOOD	Xenobiotics TRAnsfer in the primary FOOD chain

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I. Introduction

I.1 Food safety, chemicals and public health

Food safety is – besides health, nutrition and food security – one of the four main goals established in the European second action plan for food and nutrition policy of the World Health Organisation (WHO). According to this action plan, efficient food safety control relies on proper systems for the monitoring and surveillance of microbial and chemical hazards at various steps in the food chain, the so called farm-to-fork approach. Hazards include pesticide residues, environmental contaminants, naturally occurring toxicants, medicine residues, antimicrobial resistance, use of antimicrobial agents in animals as well as the occurrence of radioactive isotopes (WHO, 2008).

Chemical substances can be present in food for many different reasons and the assessment of their potential impact on human health is rather complex. Food additives, for example, extend the shelf life of a food product and food colours and flavourings make food products more attractive to people. Chemicals can also be pharmacologically active and therefore used to prevent diseases in farm animals and on crops. Food contaminants on the other hand, are chemical substances that have not been intentionally added to foodstuffs and that enter the food chain via several types of routes. Dioxins and heavy metals for instance, enter the food chain as a result of environmental transfer via air, water and/or soil. Besides environmental contamination, foods may also be polluted with chemicals due to cultivation practices or during production, transport and storage (European Commission, 2014a; WHO, 2000a). Some examples of this type of contamination are the formation of acrylamide in starchy foods during heating at high temperatures, the production of botulinum toxin by *Clostridium botulinum* in inadequately processed canned foods and the migration of benzophenone – an ink component – from cardboard to food products during frozen storage (Johns et al., 2000; Medeiros Vinci et al., 2012; WHO, 2000a).

Since contamination generally has a negative impact on the quality of food and may imply a risk to human health, the European Commission has taken measures to minimise the presence of chemical substances in foodstuffs. For instance, basic principles for contaminants in food have been laid down in the European Council Regulation 315/93/EEC and its amendments (Official Journal of the European Union, 1993a). This legislation states among others that food products shall not be placed on the European market if they contain a contaminant to an amount that is unacceptable from the public health viewpoint and in particular at a toxicological level. Furthermore, contaminant levels should be kept as low as possible following recommended good working practices. In Commission Regulation (EC) No 1881/2006 and its amendments, maximum residue levels even have been set for the following contaminants: mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, fumonisins, T-2/HT-2 toxin, citrinin and patulin), metals (cadmium, lead, mercury and inorganic tin), dioxins, dioxin-like polychlorinated biphenyls (PCBs) and non-dioxin-like PCBs, polycyclic aromatic hydrocarbons (PAHs; benzo(a)pyrene, benz(a)antracene, benzo(b)fluoranthene and chrysene), 3-monochloropropane-1,2-diol (3-MCPD), melamine, erucic acid and nitrate (Official Journal of the European Union, 2006a).

This PhD dissertation will focus on the presence and related human exposure of phthalates in food. Phthalates are a group of organic, lipophilic chemical substances that can enter the food chain via the environment as well as via migration from contact materials. In the next chapters of this introduction, an overview is given of the physico-chemical properties, user applications and

environmental fate of phthalates, their main exposure routes and possible health effects in humans, existing European legislation about phthalates and possible contamination pathways for these substances in foods. Methods to assess both integral and dietary human exposure to phthalates are also discussed. The introduction ends with the outline and objectives of this PhD thesis.

I.2 Physico-chemical properties, user applications and environmental fate of phthalates

Phthalates is the common generic name for dialkyl or alkyl aryl esters of *ortho*-phthalic acid (1,2-benzene dicarboxylic acid). The general chemical structure of this group of substances is depicted in Figure 1. To date, more than 30 different phthalate compounds are commercially available on the market (Cao, 2010; David et al., 2003). Eight of them are considered in this dissertation, namely dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), dicyclohexyl phthalate (DCHP), di(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP).

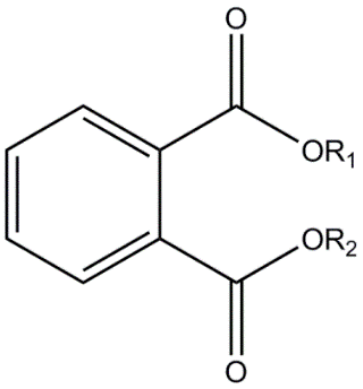

Figure 1: General chemical structure of phthalates; R1 and R2 are the same or different alkyl or aryl groups.

The behaviour of phthalates in the environment and in biological systems largely depends on the physico-chemical properties of the individual compounds. Table 1 summarises the most important characteristics of the eight considered phthalates. Data were mainly taken from European Union risk assessment reports (ECB, 2004; 2007; 2008), from the Environmental Chemistry Handbook on Phthalate Esters (Staples, 2003) and from the review of Cao (2010). As can be noticed from Table 1, most phthalates are liquids at ambient temperature: boiling points lie between 284 and 399 °C and melting points vary from -69 to 66 °C. The vapour pressure of phthalates generally declines with increasing molecular mass or alkyl chain length. A similar decreasing trend with increasing alkyl chain length is shown for the solubility in water: DMP dissolves the best in water while DEHP is rather water-insoluble. On contrary, Log(K_{ow}) values generally increase with increasing alkyl chain length. The K_{ow} parameter is the ratio of a chemical's equilibrium concentration in an octanol water system and is typically used to predict the partitioning of a chemical between water and animal/plant lipids or between water and sediment/soil organic matter (Cousins et al., 2003). Thus, long alkyl chain phthalates are generally more lipophilic and will adsorb stronger to organic matter than phthalates with a shorter alkyl chain length. Furthermore, according to the classification system for organic pollutants of the WHO (1989), most phthalates are categorised as semi-volatile[1] organic compounds.

[1] According to WHO (1989), semi-volatile organic compounds are compounds with a boiling point range of 240-260 °C to 380-400 °C. Polar compounds appear at the higher end of this range.

Table 1: Physico-chemical properties of the eight considered phthalate compounds.

Name	Abbre-viation	Formula	CAS No.	Molecular mass (g/mol)	Boiling point (°C)	Melting point (°C)	Vapour pressure (Pa)	Water solubility (mg/L)	Log K_{ow} (-)	Reference
dimethyl phthalate	DMP	$C_{10}H_{10}O_4$	131-11-3	194.2	284	5.5	2.2 E-1	4248	1.61	(Staples, 2003)
diethyl phthalate	DEP	$C_{12}H_{14}O_4$	84-66-2	222.2	299	-40	2.2 E-1	912	2.32	(Staples, 2003)
diisobutyl phthalate	DiBP	$C_{16}H_{22}O_4$	84-69-5	278.4	297	-58	3.2 E-1	20	4.11	(Staples, 2003)
di-*n*-butyl phthalate	DnBP	$C_{16}H_{22}O_4$	84-74-2	278.4	340	-69	9.7 E-3	10	4.57	(ECB, 2004)
benzylbutyl phthalate	BBP	$C_{19}H_{20}O_4$	85-68-7	312.4	370	-35	1.1 E-3	2.8	4.84	(ECB, 2007)
dicyclohexyl phthalate	DCHP	$C_{20}H_{26}O_4$	84-61-7	330.4	399	66	6.1 E-4	4.0	3.50	(Cao, 2010)
di(2-ethylhexyl) phthalate	DEHP	$C_{24}H_{38}O_4$	117-81-7	390.6	385	-55	3.4 E-5	0.003	7.50	(ECB, 2008)
di-*n*-octyl phthalate	DnOP	$C_{24}H_{38}O_4$	117-84-0	390.6	386	-25	6.0 E-4	0.02	8.06	(Staples, 2003)

In Europe, about one million tonnes of phthalates are produced every year. Among them, DEHP, diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) are the most dominant ones. The use of phthalates is very widespread and the type of application mostly depends on the length of the alkyl chain. A summary of the most important user applications of the eight considered compounds is given in Table 2. In general, a distinction is made between short (one up to six carbon atoms) and long (more than six carbon atoms) alkyl chain phthalates (ECPI, 2010). Short alkyl chain phthalates such as DMP, DEP, DiBP, DnBP, BBP and DCHP are used as solvents and can be present in adhesives, varnishes, resins, printing inks, personal care products, pharmaceuticals, pesticides, food contact applications, and so on. Long alkyl chain phthalates like DEHP and DnOP are mainly used as plasticiser to soften polyvinyl chloride (PVC). Products that may contain these substances are, e.g. furniture, building materials, gloves, food contact materials and medical devices (Cao, 2010; CDC, 2009; Fromme et al., 2007a; Hauser and Calafat, 2005; Kamrin, 2009; Teil et al., 2006).

Table 2: Overview of the most important user applications of phthalates.

Compound	Applications
Short alkyl chain phthalates	
DMP	Plastics (cellulose acetate butyrate), insect repellents, solid rocket propellant
DEP	Personal care products (perfumes, lotions, cosmetics, deodorants, shampoos), coatings (pharmaceuticals, cookware), pesticides, plastics (cellulose acetate)
DiBP	Coatings (pharmaceuticals, cookware), printing inks, adhesives, industrial solvents, pesticides
DnBP	Plastics (cellulose acetate), adhesives, varnishes, epoxy resins, dyes, printing inks, coatings (pharmaceuticals, cookware), regenerated cellulose, industrial solvents, pesticides
BBP	Vinyl products (floorings, tiles, gloves), adhesives, sealants, car care products, food contact materials (coatings of cookware), artificial leather, industrial solvents, regenerated cellulose
DCHP	Regenerated cellulose, printing inks, stabiliser (rubbers, resins)
Long alkyl chain phthalates	
DEHP	Plastics (PVC), flooring and wall coverings, furniture, food contact materials (coatings of cookware, gaskets in metallic caps), medical devices (blood storage bags, coatings of pharmaceuticals), printing inks
DnOP	Building materials, vinyl products (gloves, hoses), cements

Phthalates are not covalently bound in the products they are used in. As a consequence, they are constantly being released into the environment by direct release, migration, evaporation, leaching and/or abrasion during their user applications (Wittassek et al., 2011; Wormuth et al., 2006). The occurrence of phthalates in environmental media (i.e. air, soil, water and sediment) was predicted by

Cousins et al. (2003). Under equilibrium, steady-state conditions with no reaction, most phthalates are estimated to be present in soil, sediment or water with over 99% being distributed to these three media. Due to their low vapour pressures (Table 1), less than 1% will partition to air. Phthalates with more than five carbon atoms in their alkyl chain will almost exclusively be present in the organic matter of soils and sediments whereas phthalates with a shorter alkyl chain length (i.e. up to 4 carbon atoms) will mostly be present in water. For instance, estimated distribution percentages of DMP are 0.2% in air, 96.3% in water, 3.5% in soil and 0.1% in sediment. For DEHP on the other hand, predicted distribution percentages are <0.1% in air, <0.1% in water, 97.8% in soil and 2.2% in sediment.

I.3 Possible contamination pathways for phthalates in the food chain

The British Ministry of Agriculture Fisheries and Food (MAFF UK, 1995) determined DEHP and DnBP levels in 31 different food items packed in paper and cardboard. DEHP was present in 30 food samples and DnBP was detected in 27 samples with concentrations varying between 0.1 and 25 mg/kg for DEHP and between 0.04 and 62 mg/kg for DnBP. The highest concentrations were observed in meat stock, biscuits, vegetable oil and vegetable burgers. Since these phthalates are lipophilic, MAFF UK (1996) decided to conduct a follow-up survey investigating only food products that contribute most to the daily fat intake of humans. Poultry was shown to be responsible for 35% of the daily phthalate exposure via (fatty) food intake, fresh meat for 25%, eggs for 15% and dairy products for 10% (MAFF UK, 1996). In 2013, another British study (Bradley et al., 2013b) was set up in order to analyse phthalate levels in foods. In total, 119 food categories were combined into 20 pooled samples. DEHP was present in the highest concentrations (up to 0.8 mg/kg), followed by DiBP (up to 0.08 mg/kg) and DnBP (up to 0.03 mg/kg). Highest DEHP, DiBP and DnBP levels were found in fish, cereals and nuts, respectively. Based on these British studies conducted during the last two decades, it can be concluded that, although concentrations have decreased in general, the range of phthalate containing food products is still quite broad. Perhaps, (a combination of) several parameters (is) are responsible for the contamination of food items with phthalates. In the next paragraphs, potential contamination pathways for phthalates in the food chain are discussed.

Various investigations have revealed that – due to their lipophilic character – phthalates are mainly present in high-fat foods (Castle et al., 1989; Castle et al., 1990; Fankhauser-Noti et al., 2006; Sharman et al., 1994; Tsumura et al., 2002a; Zhu et al., 2010). Additionally, the presence of phthalates in foods does not only seem to depend on the fat content of a food product, but also on the amount of free fat particles (i.e. fat particles that are easily extracted from a food product by using a non-polar solvent like hexane) in a food (Eller and King, 1996; Fankhauser-Noti et al., 2006; Nehring, 2006).

Phthalates can also enter food products as a result of environmental contamination. For instance, Yin et al. (2003) revealed that paprika can be contaminated with DnBP via soil (pore water) through root uptake. Other possible plant uptake processes for phthalates from soil are diffusion into roots and adherence of splashed soil particles, followed by diffusion into plant tissues. Plants may also be contaminated with phthalates via the atmosphere. Processes from air include diffusive (gaseous) exchange with air and wet and dry particle deposition from air on plant surfaces followed by diffusion into plant tissues (Meneses et al., 2002; Samsoe-Petersen et al., 2003; Trapp and Legind, 2011). Plants and soil contaminated with phthalates can be taken up by animals. Consequently, phthalates also enter animal products due to environmental transfer.

Phthalate contamination in foods also occurs via migration from contact materials that are used during cultivation, transport, production or during preparation at home or outdoors (Dickson-Spillmann et al., 2009; Nehring, 2006). For example, a Chinese study indicated that vegetables take up DEHP from plastic mulch films that are used during field cultivation. Such mulch films contain about 16.5% DEHP and are mainly used to reflect sunlight in glasshouses (Du et al., 2009). Studies also demonstrated that milk and dairy products are contaminated with phthalates (especially DEHP) during transport due to the use of flexible milking tubes (Castle et al., 1990; Feng et al., 2005;

Sharman et al., 1994). Phthalate containing materials can also occur in the production phase. Examples are flexible pipelines, lubricants and conveyor belts (Nehring, 2006; Page and Lacroix, 1992; Tsumura et al., 2001a). Research has also indicated that heat treatment steps (e.g. frying, deep-frying, pasteurisation and sterilisation) during the production process of a foodstuff enhance migration (Castle, 2007; Pedersen et al., 2008; Tsumura et al., 2001b). Finally, an example of phthalate containing materials used during preparation is PVC gloves. Based on the surveys of Tsumura et al., it was demonstrated that phthalates migrated from PVC gloves during the preparation of ready-to-eat meals from shops, factories and restaurants (2001b) and from hospitals (2001a).

Packaging is a special type of food contact material, since food products are usually stored in it for extended periods before they are consumed. In general, the longer a foodstuff is stored in a phthalate containing packaging, the higher the risk that phthalates actually migrate into the food (Castle, 2007). Furthermore, a higher surface to volume ratio enhances migration, because a larger part of the food product comes into contact with the packaging material (Nehring, 2006).

The use of phthalates in packaging materials is very widespread. For instance, phthalates are added to PVC plastisols with typical concentrations between 25 and 40% by weight. These plastisols are used in the metal lids of glass jars to create a hermetic sealing (Leadbitter, 2003; Pedersen et al., 2008). Migration of phthalates from metal lids has already been confirmed by various researchers (Fankhauser-Noti et al., 2006; Fankhauser-Noti and Grob, 2006; Pedersen et al., 2008). Cling films made from PVC, regenerated cellulose or cellulose acetate used to wrap foods can also contain phthalates (Cao, 2010). Another application is the use of phthalates in printing inks and adhesives (Cao, 2010; Castle et al., 1989; Nerin et al., 1993). Since most packaging types (with the exception of metal and glass) are (semi)permeable, phthalates can migrate from inks or adhesives through the packaging into food products (Aurela and Söderhjelm, 2007; Castle et al., 1989; Nerin et al., 1993). Finally, also recycled packaging materials are potential contamination sources for phthalates in foods due to traces of phthalates from previous applications (Cao, 2010; von Wright, 2007).

I.4 Human exposure to phthalates and possible health effects

As a consequence of their widespread use (see Section I.2), people are extensively and continuously exposed to phthalates via different exposure routes. This chapter explains the most important exposure pathways for phthalates and their metabolic fate in the human body. Health effects commonly associated with phthalate exposure are discussed in this chapter as well.

I.4.1 Exposure pathways for phthalates in humans

I.4.1.1 Oral ingestion

Oral ingestion is the principal exposure pathway for the entry of compounds that are present in foods and drinks (WHO, 2000a). Specifically for phthalates, numerous studies have indicated that, for the general population, dietary intake is the most important exposure route, especially for DEHP, DiBP and DnBP (Clark et al., 2003a; Fromme et al., 2007b; Wittassek et al., 2011; Wormuth et al., 2006). Even consumers with a high risk awareness of chemicals in foods or who try to make conscious food choices (e.g. purchasing organic foods or consuming more healthy foods) cannot avoid dietary exposure to phthalates. As an example, a Swiss-German study (Dickson-Spillmann et al., 2009) revealed that conscious customers are actually more exposed to DEHP, BBP and DEP through their diet compared to others. In this study, higher exposure rates in the conscious customer group were probably due to relatively high phthalate concentrations in wholemeal bread and cereals. Similarly, in an American survey (Sathyanarayana et al., 2013), urinary DEHP metabolite concentrations significantly increased among participants who had completely modified their dietary pattern (i.e. only consuming fresh and organic, catered foods prepared without plastics) for five days in order to reduce exposure to phthalates.

To test the hypothesis that food is a major exposure route, the effect of fasting on the body burden to phthalates was examined in a German survey (Koch et al., 2013). In this study, five adults only drank glass-bottled mineral water for two days. Urine samples of these persons were collected before, during and after the fasting period. Urinary metabolite levels of DEHP, DiDP and DiNP decreased rapidly within the first day, suggesting that food products were by far the dominant exposure pathway for these compounds. On the other hand, for DMP, DEP, DnBP, DiBP and BBP, the effect of fasting was less pronounced indicating that other non-food exposure sources were still significantly contributing during the fasting study.

Ingestion of dust and soil is considered to be the second major pathway of exposure for phthalates. For instance, infants and toddlers are known to incidentally ingest small amounts of dust and soil by mouthing hands. For this age group (0-3 years old), exposure through dust ingestion contributes for more than 70% to the total daily BBP exposure (Wormuth et al., 2006). Besides BBP, DEHP is also considered to be omnipresent in household dust (Rudel et al., 2003). A Swedish study pointed out that this may be related to the presence of PVC walls and floorings in houses (Bornehag et al., 2004; 2005).

Mouthing objects is another route of exposure to phthalates, especially for young children. During sucking or biting on plasticised toys, dummies, milk bottles, etcetera, phthalates may be released and ingested by infants and toddlers (RIVM, 1998; Stringer et al., 2000; Wormuth et al., 2006). For

instance, mouthing is responsible for 8-9%, 55-82% and more than 90% of the total exposure to DEHP, DiDP and DiNP, respectively (Wormuth et al., 2006).

A specific exposure route for DEP and DnBP is the intake of enteric-coated tablets. This type of medication allows the release of active ingredients into the small intestine or in the colon. Enteric coatings may contain several milligrams of phthalates per capsule. After adults had taken coated medications, significantly higher metabolite levels of DEP and DnBP were observed in their urine (Fromme et al., 2007a; Hauser et al., 2004; Hernández-Díaz et al., 2009; 2013; Seckin et al., 2009).

Phthalates that are ingested, enter the body by absorption in the gastrointestinal tract. According to Wormuth et al. (2006), average absorption rates are about 55-82% depending on the phthalate compound. In principle, absorption of phthalates can take place anywhere along the digestive tract, i.e. from the mouth to the rectum. However, the major site for absorption is the small intestine because of its physiological function of absorbing nutrients (WHO, 2000a).

I.4.1.2 Inhalation

The main lung function is to control the exchange of oxygen from air to blood and of carbon dioxide from blood to air. For this purpose, alveolar walls are very thin and do not only allow the passage of oxygen, but also of many other chemical substances (WHO, 2000a). Specifically for phthalates, uptake rates via inhalation are currently unknown. In the European risk assessment reports for phthalates, uptake rates of 100 and 75% are proposed for children and adults, respectively (ECB, 2003a; 2003b; 2004; 2007; 2008). Once inhaled and absorbed through the lungs, phthalates may directly affect the respiratory as well as the cardiovascular system (WHO, 2000a).

Inhalation is a dominant exposure pathway for volatile short alkyl chain phthalates (Rudel and Perovich, 2009). For instance, Wormuth et al. (2006) calculated for the general population that 70 to almost 100% of the total exposure to DMP originates from inhalation. In an American study, DEP and DnBP were identified as the most abundant phthalate compounds in indoor air (Rudel et al., 2003).

Comparing concentrations of phthalates in indoor and outdoor environments reveals that in general, indoor air is more contaminated than ambient air, which can be explained by the use of phthalates in various household products and building materials (Rudel et al., 2003; Rudel and Perovich, 2009). In cars and other transportation vehicles, phthalate concentrations in air can also be relatively high due to the evaporation of phthalates from PVC interiors (Geiss et al., 2009). Moreover, it has been demonstrated that a higher room temperature increases the concentrations of phthalates in the indoor environment (Wensing and Salthammer, 2005). In the outdoor environment, urban and suburban concentrations are generally higher than rural and remote concentrations (Rudel and Perovich, 2009). According to Rakkestad et al. (2007), outdoor sources like the wearing of tires are responsible for this phenomenon.

The use of sprays (spray paints, hair sprays, pesticides, and so on) can be another exposure pathway for phthalates by generating aerosols which are inhaled by consumers. Mostly, sprays are used infrequently, although some people are occupationally exposed to these products. For instance, in an English study, occupational exposure to hair spray was correlated with higher phthalate exposure levels (Ormond et al., 2009).

I.4.1.3 Dermal absorption

When a chemical penetrates the skin, its toxicity depends – just like for oral ingestion and inhalation – on the degree of absorption in the blood that takes place (WHO, 2000a). According to Wormuth et al. (2006), phthalates' dermal absorption rates are very low, i.e. 0.1-2.0% for adults and 1.3-4.1% for children. Once penetrated, phthalates enter the blood stream and are then carried to all parts of the human body (WHO, 2000a).

Consumers can be in dermal contact with a diversity of products that may contain phthalates. The duration of contact with these products can be long (e.g. clothes, boots and toys) or rather short (e.g. paints and gloves). Furthermore, people can also be exposed to small amounts of phthalate containing dust or soil that remains on their hands (Müller et al., 2003; Wormuth et al., 2006).

Of all consumer products, personal care products seem to account for most of the dermal exposure to phthalates. For instance, up to 80% of the daily exposure to DEP is caused by the use of products such as lotions, shampoos, perfumes, deodorants, etcetera (Wormuth et al., 2006). In a study conducted on 163 American infants, urinary metabolite concentrations of DEP and DMP were significantly associated with the application of baby lotion, DiBP metabolite concentrations with the use of baby powder and DMP metabolite concentrations with the use of baby shampoo (Sathyanarayana et al., 2008). Associations appear to increase with the number of personal care products that are used (Duty et al., 2005a; Romero-Franco et al., 2011; Sathyanarayana et al., 2008). In contrast, for some phthalates, the use of personal care products is associated with lower phthalate metabolite levels in urine. This remarkable observation has been shown in the study of Duty et al. (2005a) for the primary metabolites of DnBP, BBP and DEHP in urine samples of American adult men and in the second Flemish Environment and Health Study (FLEHS II) for the metabolites of DnBP and BBP in the urine of Flemish adolescents (Geens et al., 2014). According to Duty et al. (2005a), a reason for these inverse relationships is unknown, although several hypotheses could be plausible. For instance, Duty et al. (2005a) suggested that other ingredients in personal care products might act as a barrier to the absorption of DnBP, BBP and DEHP.

I.4.1.4 Parenteral exposure from medical devices

People under medical treatment are a sensitive subpopulation with regard to phthalate exposure. For instance, some studies have demonstrated that blood bags and tubings are – in addition to food ingestion – an important source for DEHP exposure (FDA, 2001; Health Care Without Harm, 2009). Higher DEHP metabolite concentrations have been observed in the urine of neonatal intensive care unit infants, dialysis patients, blood donors, and so on (Calafat et al., 2004; Frederiksen et al., 2007; Koch et al., 2005b; 2005c; Weuve et al., 2006).

Recently, Monfort and co-workers (2010; 2012) have suggested analysing urinary DEHP metabolites in athletes as a screening measure for illicit blood doping. The logic behind this recommendation is that elevated urinary DEHP metabolites can indicate a recent blood transfusion, which is hard to detect or prove otherwise.

I.4.2 Metabolic fate of phthalates in the human body

Phthalates are usually metabolised in at least two steps, i.e. a phase I hydrolysis followed by a phase II conjugation step (Figure 2). Once entered in the human body, phthalate diesters are first rapidly hydrolysed into primary phthalate metabolite monoesters. This process is catalysed by lipases and esterases and occurs in the intestines (mainly the small intestine) and lung parenchyma. Unlike for most chemicals, this metabolic step is not a detoxification step, since studies have shown that phthalate monoesters are more bioactive than their parent compounds. In a second step, the remaining alkyl chain of the monoester can be metabolised via hydroxylation or oxidation to secondary metabolites. Both the hydrolytic monoester and the secondary metabolites can be conjugated with glucuronic acid to form hydrophilic phase II glucuronide conjugates. This last transformation step is often catalysed by the enzyme uridine 5'-diphosphoglucuronyl transferase (Frederiksen et al., 2007; Koch and Calafat, 2009; Wittassek et al., 2011; 2006). Relatively polar and short alkyl chain phthalates are primarily metabolised into their hydrolytic monoesters, while the monoesters of long alkyl chain phthalates are usually further metabolised to more hydrophilic, oxidised products (Hauser and Calafat, 2005).

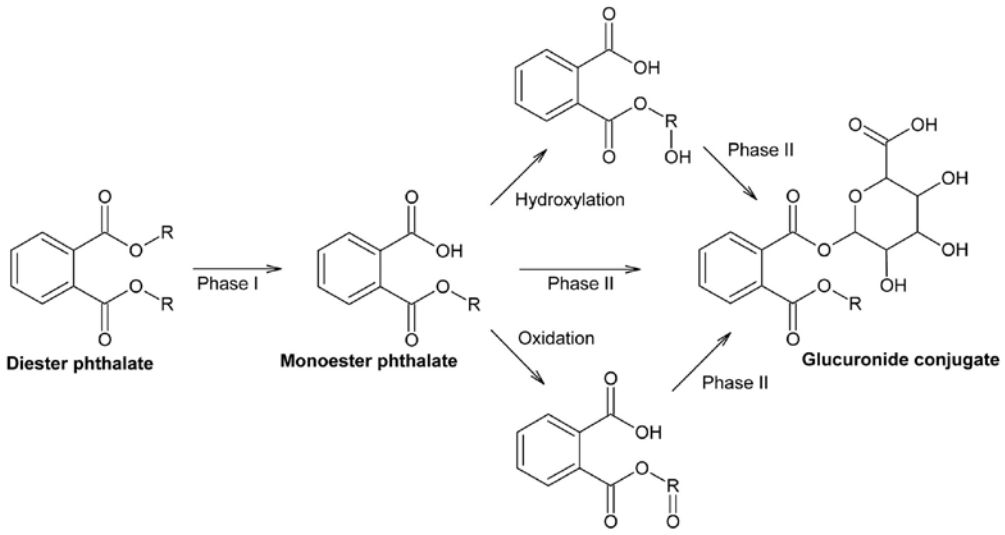

Figure 2: Metabolic pathways for phthalates (Frederiksen et al., 2007; with permission).

Phthalates are mainly excreted via urine; only a small amount is eliminated via faeces (Frederiksen et al., 2007; Hauser and Calafat, 2005; Koch and Calafat, 2009). For instance, a German study figured out that about 67% of DEHP was excreted in the urine of a male volunteer after 24h of oral ingestion. On the second day, an additional 3.8% of the DEHP dose was excreted (Koch et al., 2005a). Similar results were obtained for DnBP in rats: within 48h, 80-90% of orally administrated DnBP was metabolised and excreted in urine, while only 5% was excreted in faeces (study cited in Frederiksen et al., 2007). In Table 3, an overview is given of the primary and secondary metabolites of the eight considered phthalates, that are often used as urinary biomarker (Koch and Calafat, 2009). When monoesters and oxidative metabolites undergo phase II biotransformation to produce glucuronide conjugates, their water solubility increases and consequently, their urinary excretion also increases (Hauser and Calafat, 2005).

Table 3: Overview of primary and secondary phthalate metabolites often used as urinary biomarker.

Parent compound	Primary metabolite	Secondary metabolite
Short alkyl chain phthalates		
DMP	Mono-methyl phthalate (MMP)	n.a.
DEP	Mono-ethyl phthalate (MEP)	n.a.
DiBP	Mono-isobutyl phthalate (MiBP)	3OH-mono-methylpropyl phthalate (OH-MiBP)
DnBP	Mono-*n*-butyl phthalate (MnBP)	3OH-mono-*n*-butylphthalate (OH-MnBP) 3carboxy-mono-propyl phthalate (3cx-MPP or MCPP)
BBP	Mono-benzyl phthalate (MBzP)	n.a.
DCHP	Mono-cyclohexyl phthalate (MCHP)	n.a.
Long alkyl chain phthalates		
DEHP	Mono(2-ethylhexyl) phthalate (MEHP)	5OH-mono(2-ethylhexyl) phthalate (5OH-MEHP or MEHHP) 5oxo-mono(2-ethylhexyl) phthalate (5oxo-MEHP or MEOHP) 5carboxy-mono(2-ethylhexyl) phthalate (5cx-MEPP or MECPP)
DnOP	Mono-*n*-octyl phthalate (MnOP)	n.a.

n.a.: not available.

Specifically for Belgium, the presence of phthalate metabolites has been investigated in Flemish adolescents during "FLEHS II" (Geens et al., 2014) and in Belgian children and their mothers during the "DEMOCOPHES" project (The Belgian Steering Committee on HBM, 2013). Table 4 summarises the average values of the urinary phthalate metabolites observed in these two studies. For comparison, average urinary phthalate metabolite levels from European children and their mothers from the DEMOCOPHES project (FOD, 2013a) and from the national German and American biomonitoring studies "GerES IV" (Becker et al., 2009) and "NHANES" (CDC, 2009), respectively, were added to Table 4 as well.

Since a couple of years, more and more research groups have investigated the occurrence of phthalates in human breast milk, because this medium is an additional dietary exposure source for phthalates in newborns and infants. Table 5 gives an overview of some recent studies (Fromme et al., 2011; Högberg et al., 2009; Latini et al., 2009; Main et al., 2006) in which phthalate metabolites have been analysed in human breast milk. Unfortunately, for now, the presence of phthalates in Belgian breast milk has not been examined yet.

Table 4: Urinary phthalate metabolite levels investigated in several national biomonitoring surveys.

	FLEHS II	**DEMOCOPHES**				**GerES IV** [b]	**NHANES**		
Reference	(Geens et al., 2014)	(The Belgian Steering Committee on HBM, 2013)				(Becker et al., 2009)	(CDC, 2009)		
Country	Flanders (Belgium)	Belgium	Belgium	Europe	Europe	Germany	USA	USA	USA
Sampling period	2008-2009	2011-2012	2011-2012	2011-2012	2011-2012	2003-2006	2003-2004	2003-2004	2003-2004
Population	Adolescents	Children	Mothers	Children	Mothers	Children	Children	Adolescents	Adults
Age category	14-15 yr.	6-11 yr.	≤ 45 yr.	6-11 yr.	≤ 45 yr.	3-14 yr.	6-11 yr.	12-19 yr.	≥ 20 yr.
No. of participants	206	125	125	1844	1844	599	342	729	1534
Average urinary concentration (µg/l) of									
MEP	-	26	36	34	48	-	95	225	205
MnBP	39	39	31	35	24	93	36	27	19
MiBP	-	58	3	45	30	88	6.6	4.6	3.5
MBzP	32	8.8	6.5	7.1	4.5	18	34	22	11
DEHP metabolites [a]	51	36	21	48	29	89	65	51	35

[a] Sum of MEHP, MEHHP and MEOHP; [b] Median instead of average concentrations.

Table 5: Phthalate metabolites analysed in human breast milk samples.

	Main et al., 2006 [a]		**Högberg et al., 2008**	**Latini et al., 2009** [a]	**Fromme et al., 2011**
Country	Denmark	Finland	Sweden	Italy	Germany
Sampling period	1997-2001	1997-2001	2001	2006	2007-2008
Population	Women	Women	Women	Women	Women
Age category	21-40 yr.	21-40 yr.	23-39 yr.	18-41 yr.	22-40 yr.
No. of participants	65	65	42	62	78
Average breast milk concentration (µg/l) of					
MEP	0.9	1.0	2.5	-	-
MnBP	4.3	12	1.2	1.5	2.6
MiBP	-	-	1.3	19	14
MBzP	0.9	1.3	0.6	<0.3	-
MEHP	9.5	13	1.3 [b]	8.4 [d]	3.0

[a] Median instead of average concentrations.

I.4.3 Health effects related to phthalate exposure

Some phthalates and their metabolites are suspected to be endocrine disrupting compounds (EDCs). EDCs are exogenous substances or mixtures that alter the functioning of the endocrine system and consequently cause adverse health effects in intact organisms, or their progeny, or (sub)populations. In Europe, a priority list was made of all chemicals with potentially endocrine disrupting activities. Based on the strength of evidence for endocrine disruption, chemicals were subdivided into three categories: Category 1 - evidence of endocrine disrupting activity in at least one species using intact animals; Category 2 - at least some *in vitro* evidence of biological activity related to endocrine disruption; Category 3 - no evidence of endocrine disrupting activity or no data available. The phthalates DEP, DnBP, BBP, DCHP and DEHP have been classified as Category 1 substances, DiBP, DiNP and DiDP as Category 2 and DnOP as Category 3 (European Commission, 2014b). Although available literature data regarding the toxicology of EDCs (and phthalates) are often inconsistent with each other, there is no scientific doubt that exposure to this type of chemicals cause negative health effects, even at background doses (Grandjean et al., 2006; Superior Health Council, 2013; see also Chapter IV.2).

Health outcomes associated with phthalates have been mostly examined in *in vitro* and experimental animal studies. For instance, animal surveys (mainly on rodents) reported that exposure to phthalates such as DnBP, BBP and DEHP causes a decline in foetal testosterone and insulin-like growth factor-3 resulting in a syndrome of male reproductive abnormalities, often referred to as the "phthalate syndrome". Furthermore, laboratory surveys have shown that phthalates exhibit marked differences in toxicity depending on their structure and – as already stated in Section I.4.2 – that the monoester phthalate metabolites are generally more toxic than the parent diester compounds (Hauser and Calafat, 2005; Swan, 2008).

In this section, an overview is given of potential adverse health effects of phthalates in humans. The majority of the described effects are derived from epidemiological studies and – unless mentioned otherwise – are dealing with background exposure to phthalates. A distinction is made between effects observed in children and in adults. At the end of this section, Table 6 summarises potential health effects per phthalate compound.

I.4.3.1 Health outcomes in children

a) Gestational age and birth weight

The effects of phthalate exposure on gestational age (i.e. the estimated age of a fetus expressed in weeks, calculated from the first day of the last normal menstrual period) and birth weight were investigated by Latini et al. (2003) by measuring serum levels of MEHP in cord blood of 84 Italian infants. Newborns having MEHP detected in their cord blood, had a significantly lower gestational age compared with other infants. No significant relationship was found between MEHP and birth weight. In a Taiwanese study, prenatal exposure to phthalates and its effect on birth weight and gestational age were also examined. MnBP, MEHP, MEP, MBzP and MMP levels were determined in the amniotic fluid of 64 pregnant women. For female newborns, a significant positive association was found between MnBP concentration in amniotic fluid and birth weight. The gestational age of the newborns was not influenced by prenatal exposure to any of the five considered phthalate compounds (Huang et al., 2009).

b) Anogenital distance

In humans, the anogenital distance (AGD; i.e. the physical distance between anus and the genitalia) in males is normally about twice that in females (Salazar-Martinez et al., 2004). This difference is a direct reflection of growth stimulating actions of androgens, such as testosterone, on the perineum in foetal life. In other words, AGD is an indicator of the level of androgen action in the foetus and thus of the masculinisation process (Sharpe, 2005).

Some epidemiological studies suggest that prenatal exposure to a number of phthalates at environmental levels might affect the AGD. For instance, Swan et al. (2005) examined AGD and other genital measurements in male infants in relation to their mothers' phthalate exposure. MEP, MnBP, MBzP and MiBP concentrations in prenatal maternal urine samples were associated with a shortened AGD. Of the three investigated DEHP metabolites, MEHP was not affecting the AGD while MEOHP and MEHHP were borderline significant in affecting the AGD. MMP was also not associated with a shortened AGD. A few years later, the results of this study were updated, i.e. more participants were included and statistical analyses were improved (Swan, 2008). In the new dataset, urinary concentrations of MEP, MnBP, MEHP, MEOHP and MEHHP in male infants were significantly and inversely related to AGD; MBzP was no longer associated with AGD. Furthermore, a significantly negative correlation between MnBP levels in amniotic fluid and the AGD in female newborns has been observed by Huang et al. (2009).

c) Cryptorchidism and hypospadias

Cryptorchidism (undescended testes) and hypospadias (abnormally placed urethra) are the two most common congenital malformations in male newborns (with 2-4% and 0.3-0.7% incidence, respectively). Both malformations are symptoms of the testicular dysgenic syndrome or "phthalate syndrome" and thus may be caused by phthalate exposure (Sharpe, 2005).

The difference in prevalence of congenital cryptorchidism in infants between two North-European countries (Denmark and Finland) was investigated by Boisen and co-workers (2004). Prevalence of cryptorchidism at birth was 9.0% in Denmark and 2.4% in Finland. Even after adjustment for confounding factors, significant geographic differences were still present. According to the authors, genetic factors could account for this geographic difference, but it would be more likely that this dissimilarity may be explained by environmental factors, including endocrine disruptors and lifestyle. Main et al. (2006) investigated whether phthalate monoester (i.e. MMP, MEP, MnBP, MBzP, MEHP and mono-isononyl phthalate (MiNP), a metabolite of DiNP) contamination of human breast milk had any influence on the difference in prevalence of cryptorchidism in this Danish-Finnish cohort study. In contrast with rodent studies, no significant difference was observed between children with or without cryptorchidism with regard to any phthalate monoester concentration in breast milk, if analysed either separately for each country or together. In fact, levels of MnBP, MBzP and MEHP were higher in Finnish than in Danish breast milk, which is the inverse compared with the prevalence of cryptorchidism in the two countries. Main et al. (2006) concluded that their study groups may have been too small to detect subtle changes related to the presence or absence of cryptorchidism and that further research is urgently needed.

The risk of hypospadias in relation to phthalate exposure was investigated in a British case-control study. In that study, a two- to threefold increased risk of hypospadias was found among children of mothers exposed to hair spray and phthalates in the workplace during pregnancy (Ormond et al., 2009).

d) Hormone production

Main et al. (2006) determined phthalate monoester metabolites in human breast milk samples that were collected postnatally after one to three months. Additionally, serum samples of male newborns were analysed for gonadotropins (follicle-stimulating hormone (FSH) and luteinising hormone (LH)), sex-hormone binding globulin (SHBG), testosterone and inhibin B. The free testosterone index was calculated from testosterone and SHBG: [(testosterone x 100)/SHBG]. MEP and MnBP were positively correlated with SHBG and MMP, MEP and MnBP with the ratio of LH to free testosterone. Besides, MnBP was inversely related with free testosterone.

e) Thelarche

Thelarche or premature breast development is the growth of mammary tissue in girls younger than eight years without any other manifestations of puberty. In a Puerto Rican case-control study, Colon et al. (2000) investigated whether exposure to phthalate compounds may be responsible for more cases of thelarche in Puerto Rican girls during the last two decades. For this purpose, they measured several phthalates and monoester metabolites in blood serum samples of 41 thelarche patients and 35 controls. Significantly higher levels of DMP, DEP, DnBP, DEHP and MEHP were analysed in thelarche patients than in controls suggesting a possible association between phthalate exposure and the cause of premature breast development in Puerto Rican girls. Although this study was noteworthy since the effect of phthalate exposure on thelarche was an understudied area, a few years after the results of this study were published, questions have been raised about the analytical procedures used in this study to analyse phthalate diesters in blood serum (Hauser and Calafat, 2005).

In a multi-ethnic longitudinal study, associations between phthalate exposure and the development of breasts and pubic hair was investigated in 6-8 years old American girls. Breast and pubic hair development were present in 30% and 22% of the girls, respectively. Small inverse associations were seen for high molecular mass phthalate metabolites (sum of MBzP, 3cx-MPP, mono-2-ethyl-5-carboxypentyl phthalate, MEHP, MEHHP and MEOHP) and the stage of pubic hair development. For low molecular mass phthalate metabolites (sum of MEP, MnBP and MiBP), a positive trend was observed for both breast and pubic hair development (Wolff et al., 2010).

f) Respiratory function, allergic symptoms and diseases

Various studies have examined respiratory function, asthma and allergy and their relation to phthalate exposure. In a Swedish case-control study, dust samples from the houses of 400 children were examined for the presence of phthalates. Of the 400 participants, 198 children suffered from persistent allergic symptoms and the 202 other children showed no allergic symptoms. Analysing the case group by symptoms revealed that BBP was associated with rhinitis and eczema, whereas DEHP was related to asthma (Bornehag et al., 2004). In another case-control survey, wheezing among

preschool children in Bulgaria was linked to higher DEHP concentrations in dust from the children's bedrooms (Kolarik et al., 2008). According to Jaakkola et al. (1999; 2008), the development of bronchial obstruction in children during the first two years of life is related to the presence in their homes of PVC flooring and textile wall materials, which are known to contain phthalates (Table 2).

g) Childhood behaviour

Children's play behaviour was investigated in order to study possible links between prenatal phthalate exposure and neurodevelopmental outcomes. Play behaviour scores were examined in relation to prenatal maternal urinary concentrations of several phthalate metabolites for boys and girls separately. Urinary concentrations of MnBP, MiBP and their sum as well as levels of MEOHP, MEHHP and the sum of MEOHP, MEHHP and MEHP were associated with a less masculine score in boys. Although based on a small sample size (74 boys and 71 girls), these results suggest that prenatal exposure to phthalates may be associated with less male-typical play behaviour in boys and that phthalates have the potential to alter androgen-responsive brain development in humans (Swan et al., 2010).

The role of prenatal phthalate exposure on child behaviour and executing functioning was tested among a multi-ethnic population in New York. Phthalate metabolites were analysed in third-trimester maternal urine samples and children were assessed three times for cognitive and behavioural development between the ages of 4 and 9 years. Several behavioural domains (e.g. aggression, conduct problems and attention problems), which are commonly found to be affected in children clinically diagnosed with conduct or attention deficit hyperactivity disorders (ADHD), were found to be positively associated with prenatal exposure to MMP, MEP, MiBP and MnBP (Engel et al., 2010).

I.4.3.2 Health outcomes in adults

a) Hormone production

Phthalates seem to influence the production of hormones in adults. BBP exposure in adult men was significantly associated with a decrease in FSH levels in serum. Additionally, exposure to DnBP was associated with an increase in inhibin B serum levels, although this association was of borderline significance (Duty et al., 2005b).

In a cross-sectional study, the effect of occupational exposure to high levels of DnBP and DEHP on the balance of gonadotropin and gonadal hormones (LH, FSH, free testosterone and estradiol) was investigated. To this end, urine and blood samples of 74 males working at a factory producing unfoamed PVC floorings were compared with samples from 63 male workers from a construction company. In comparison with the unexposed workers, workers from the PVC factory had significantly higher concentrations of MnBP and MEHP. Furthermore, free testosterone levels in serum were significantly lower in the exposed than in the unexposed work group, which suggests that free testosterone decreases significantly with an increase in phthalate metabolite levels (Pan et al., 2006).

Meeker et al. (2007) measured urinary concentrations of phthalate metabolites and serum levels of thyroid hormones in male partners of subfertile couples. In their study, they suggested that urinary MEHP concentrations may be associated with altered free thyroxine and/or total triiodothyronine levels in adult men, but that additional research is needed to confirm their findings.

b) Semen quality

According to Carlsen et al. (1992), the quality of human semen has clearly declined since 1938. This decline is probably due more to environmental rather than genetic factors. Whether exposure to phthalates influences the quality of semen in humans has been investigated by Duty et al. (2003a; 2003b). This research group found associations between urinary concentrations of MEP and increased deoxyribonucleic acid (DNA) damage in sperm as well as between MnBP and MBzP levels and reduced sperm motility. The continuation of this study has been reported by Hauser et al. (2006; 2007). In the follow-up study, more participants were included and the effect of the metabolites of DEHP on human sperm quality was also considered. Besides confirming the previous results, a relation between MEHP and sperm DNA damage was observed. Noteworthy is that the latter association was found after adjustment for the oxidative metabolites of DEHP, suggesting that the oxidation of MEHP to MEHHP and MEOHP may actually be “protective” against sperm DNA damage.

c) Anogenital distance

In a Spanish cross-sectional study, the relationship between the AGD and adult female reproductive characteristics was investigated in 100 college-age volunteers. One of the outcomes of this study was that the AGD was positively associated with the number of ovarian follicles (Mendiola et al., 2012). Although based on studies with mice instead of humans, Moyer and Hixon (2012) revealed that prenatal exposure to DEHP, among others, may be responsible for this increase in number of mature follicles.

d) Endometriosis

DEHP and MEHP levels were analysed in serum and peritoneal fluid of 24 healthy women and 35 women with endometriosis. Endometriotic women showed significantly higher plasma concentrations of DEHP than women in the control group while plasma MEHP levels were comparable between the two groups. Cases and controls had similar levels of peritoneal DEHP and MEHP concentrations (Cobellis et al., 2003). However, the correlation between serum DEHP and MEHP concentrations was weak (r=0.16), which raised questions regarding the quality of the DEHP measurements. Because peritoneal fluid may contain esterases capable of hydrolysing DEHP to MEHP, further exploration into why the relationships between DEHP and MEHP with endometriosis differed is warranted (Hauser and Calafat, 2005).

e) Respiratory function, allergic symptoms and diseases

Hoppin et al. (2004) examined the association between exposure to the primary metabolites of DnBP, BBP, DEP and DEHP in 240 American adults and four pulmonary function parameters (forced vital capacity (FVC), forced expiratory volume at 1 sec (FEV_1), peak expiratory flow (PEF) and maximum mid-expiratory flow). After adjusting for race, age, height, body mass index and smoking,

they found inverse associations between male urinary concentrations of MnBP and FVC, FEV_1 and PEF and between urinary MEP levels and FVC and FEV_1. No consistent associations were observed in women.

Recently, the association between urinary phthalate metabolites and allergic symptoms and sensitisation was investigated among the American population. MBzP was the only metabolite that was positively associated with ongoing allergic symptoms in adults (wheeze, asthma, hay fever and rhinitis). Furthermore, 3cx-MPP and the sum of DEHP metabolites were positively associated with allergic sensitisation in adults whereas MEP was inversely related to sensitisation (Hoppin et al., 2013).

f) Obesity and diabetes

Phthalate exposure and its relation to abdominal obesity and insulin resistance was examined in American adult men. Urinary concentrations of the metabolites MBzP, MEP, MEHHP and MEOHP were significantly associated with increased waist circumference. Three phthalate metabolites (MEP, MnBP and MBzP) were significantly related to the HOmeostasis Model Assessment (HOMA) index[2], which is often used in epidemiological studies as a measure of insulin resistance (IR). Although these findings have to be confirmed by longitudinal studies, this study provides preliminary evidence that exposure to phthalates may contribute to the population burden of obesity, insulin resistance and related clinical disorders (Stahlhut et al., 2007).

I.4.3.3 Health outcomes per phthalate compound

Table 6: Summary of potential health outcomes in humans grouped per phthalate compound.

Phthalate	Metabolite	Health outcome in children	Health outcome in adults
DMP	-	Thelarche ♀↑ (Colon et al., 2000)	
	MMP	Birth weight = (Huang et al., 2009)	
		Gestational age = (Huang et al., 2009)	
		AGD ♂= (Swan et al., 2005)	
		Cryptorchidism ♂= (Main et al., 2006)	
		SHBG ♂ = (Main et al., 2006)	
		[LH/free testosterone] ♂↑ (Main et al., 2006)	
		Free testosterone ♂= (Main et al., 2006)	
		ADHD ↑ (Engel et al., 2010)	
DEP	-	Thelarche ♀↑ (Colon et al., 2000)	
	MEP	Birth weight = (Huang et al., 2009)	Sperm DNA damage ♂↑ (Duty et al., 2003a; 2003b)
		Gestational age = (Huang et al., 2009)	Pulmonary function ♂ ↓ (Hoppin et al., 2004)
		AGD ♂↓ (Swan, 2008)	Pulmonary function ♀ = (Hoppin et al., 2004)
		Cryptorchidism ♂= (Main et al., 2006)	Allergic sensitisation ↓ (Hoppin et al., 2013)
		SHBG ♂↑ (Main et al., 2006)	Waist circumference ♂↑ (Stahlhut et al., 2007)
		[LH/free testosterone] ♂↑ (Main et al., 2006)	Insulin resistance ♂↑ (Stahlhut et al., 2007)
		Free testosterone ♂= (Main et al., 2006)	
		ADHD ↑ (Engel et al., 2010)	

[2] The HOMA-IR index is calculated according to the formula: (FPI x FPG)/22.5. In this equation, "FPI" is the fasting plasma insulin concentration (in mU/L) and "FPG" is the fasting plasma glucose concentration (in mmol/L) (Wallace et al., 2004).

Phthalate	Metabolite	Health outcome in children	Health outcome in adults
DiBP	MiBP	AGD ♂↓ (Swan et al., 2005) Male-typical play behaviour ♂↓ (Swan et al., 2010) ADHD ↑ (Engel et al., 2010)	
DnBP	-	Thelarche ♀↑ (Colon et al., 2000)	Inhibin B ♂↑ (Duty et al., 2005b)
	MnBP	Birth weight ♀↑ (Huang et al., 2009) Birth weight ♂ = (Huang et al., 2009) Gestational age = (Huang et al., 2009) AGD ♂↓ (Swan, 2008) AGD ♀↓ (Huang et al., 2009) Cryptorchidism ♂= (Main et al., 2006) SHBG ♂↑ (Main et al., 2006) [LH/free testosterone] ♂↑ (Main et al., 2006) Free testosterone ♂↓ (Main et al., 2006) Male-typical play behaviour ♂↓ (Swan et al., 2010) ADHD ↑ (Engel et al., 2010)	Free testosterone ♂↓ (Pan et al., 2006) Sperm motility ♂↓ (Duty et al., 2003a; 2003b) Pulmonary function ♂ ↓ (Hoppin et al., 2004) Pulmonary function ♀ = (Hoppin et al., 2004) Waist circumference ♂ = (Stahlhut et al., 2007) Insulin resistance ♂↑ (Stahlhut et al., 2007)
	3cx-MPP		Allergic sensitisation ↑ (Hoppin et al., 2013)
BBP	-	Rhinitis ↑ (Bornehag et al., 2004) Eczema ↑ (Bornehag et al., 2004) Asthma = (Bornehag et al., 2004)	FSH ♂↓ (Duty et al., 2005b)
	MBzP	Birth weight = (Huang et al., 2009) Gestational age = (Huang et al., 2009) AGD ♂= (Swan, 2008) Cryptorchidism ♂= (Main et al., 2006)	Sperm motility ♂↓ (Duty et al., 2003a; 2003b) Pulmonary function = (Hoppin et al., 2013) Allergic symptoms ↑ (Hoppin et al., 2013) Waist circumference ♂↑ (Stahlhut et al., 2007) Insulin resistance ♂↑ (Stahlhut et al., 2007)
DEHP	-	Thelarche ♀↑ (Colon et al., 2000) Rhinitis = (Bornehag et al., 2004) Eczema = (Bornehag et al., 2004) Asthma ↑ (Bornehag et al., 2004) Wheezing ↑ (Kolarik et al., 2008)	Number of ovarian follicles/AGD ♀↑ (Mendiola et al., 2012; Moyer and Hixon, 2012) Endometriosis ♀↑ (Cobellis et al., 2003)
	MEHP	Gestational age ↓ (Latini et al., 2003) Gestational age = (Huang et al., 2009) Birth weight = (Huang et al., 2009; Latini et al., 2003) AGD ♂↓ (Swan, 2008) Cryptorchidism ♂= (Main et al., 2006) Thelarche ♀↑ (Colon et al., 2000)	Free testosterone ♂↓ (Pan et al., 2006) Thyroid hormone alteration ♂↑ (Meeker et al., 2007) Sperm DNA damage ♂↑ (Hauser et al., 2006; 2007) Endometriosis ♀ = (Cobellis et al., 2003) Pulmonary function = (Hoppin et al., 2004)
	MEOHP	AGD ♂↓ (Swan, 2008) Male-typical play behaviour ♂↓ (Swan et al., 2010)	Waist circumference ♂↑ (Stahlhut et al., 2007) Insulin resistance ♂ = (Stahlhut et al., 2007)
	MEHHP	AGD ♂↓ (Swan, 2008) Male-typical play behaviour ♂↓ (Swan et al., 2010)	Waist circumference ♂↑ (Stahlhut et al., 2007) Insulin resistance ♂ = (Stahlhut et al., 2007)
	Σ		Allergic sensitisation ↑ (Hoppin et al., 2013)
DiNP	MiNP	Cryptorchidism ♂= (Main et al., 2006)	
Phthalates in general	-	Hypospadias ♂↑ (Ormond et al., 2009) Bronchial obstruction ↑ (Jaakkola et al., 1999; Jaakkola and Knight, 2008)	
	High MM	Pubic hair ♀ = (Wolff et al., 2010) Pubic hair ♀↓ (Wolff et al., 2010)	
	Low MM	Thelarche ♀↑ (Wolff et al., 2010) Pubic hair ♀↑ (Wolff et al., 2010)	

MM: molecular mass.

I.5 Legislation

As elaborated in Section I.4.3, numerous studies have indicated that phthalate compounds may be associated with adverse health effects in humans. As a consequence, authorities have established regulations regarding the use of phthalates in a wide range of applications. This chapter briefly describes the regulation of phthalates under the European REACH regulation and legislations concerning their occurrence in surface water and use in toys, childcare articles, cosmetic and personal care products, medical devices and food contact materials. Additionally, exposure limit values are summarised that will be used to assess the risks related to human exposure to phthalates.

I.5.1 The REACH regulation

REACH stands for *Registration, Evaluation, Authorisation and Restriction of Chemicals* and entered into force on June 1st, 2007 (Official Journal of the European Union, 2006b). It is a regulation, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the European chemicals industry (ECHA, 2014).

REACH specifies that industry must register all chemical substances that are either produced in or imported into the European Union in annual quantities of more than or equal to one tonne. So far, 25 different phthalates have already been registered (Table 7), and more phthalates (with an annual production volume between 1 and 100 tonnes) are expected to get registered in the years to come. Registrations by industry must contain information available on the hazards that phthalates pose to human health and the environment, information on quantities produced and imported as well as (if necessary) documentation for safe production and use (Danish EPA, 2013; ECHA, 2014).

The European Chemicals Agency (ECHA) receives and evaluates individual registrations for compliance, and the EU Member States evaluate selected substances to clarify initial concerns for human health or for the environment. As can be noticed from Table 7, several of the phthalates registered in the European Union are included in the Candidate List of substances of very high concern (SVHCs) because they are toxic to reproduction. SVHCs are carcinogens (category 1a or 1b), mutagens (category 1a or 1b), toxic to reproduction (category 1 or 2), persistent and/or bioaccumulative toxicants, or substances of equal concern (e.g. having endocrine disruption properties) (ECHA, 2014).

The authorisation procedure aims to assure that the risks from SVHCs are properly controlled and that these substances are progressively replaced by suitable alternatives while still ensuring the good functioning of the European internal market. After a two-step regulatory process, SVHCs may be included in the Authorisation List and become subject to authorisation (ECHA, 2014). After a given cut-off date, the so-called sunset date, substances on the Authorisation List may not be placed on the market anymore, unless a company has obtained authorisation for this purpose. At the moment, all uses of DEHP, DiBP, DnBP and BBP are subjected to authorisation except for the use of DEHP, BBP and DnBP in the immediate packaging of medicinal products (Table 7) (Danish EPA, 2013; ECHA, 2010; 2014; Official Journal of the European Union, 2006b; 2011a).

Table 7: Phthalates already registered by industry and importers, included in the Candidate List of SVHCs or included in the Authorisation List according to REACH (ECHA, 2014).

Registered compound	Included in Candidate List of SVHCs	Included in Authorisation List
10 – 100 tonnes per year		
- Diisopentyl phthalate	X (since 19/12/2012)	
100 – 1,000 tonnes per year		
- Diallyl phthalate		
- Disodium phthalate		
- DCHP		
- 1,2-benzenedicarboxylic acid, di-C6-10-alkyl esters		
1,000 – 10,000 tonnes per year		
- Benzyl 3-isobutyryloxy-1-isopropyl-2,2-dimethylpropyl phthalate		
- Diisotridecyl phthalate		
- Diundecyl phthalate		
- 1,2-benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13-rich		
- DEP		
- DiBP	X (since 13/01/2010)	X (sunset date: 21/02/2015)
- DnBP	X (since 28/10/2008)	X (sunset date: 21/02/2015) [a]
- BBP	X (since 28/10/2008)	X (sunset date: 21/02/2015) [a]
- Diundecyl phthalate, branched and linear		
- 1,2-benzenedicarboxylic acid, di- C9-11-branched and linear alkyl esters		
- 1,2-benzenedicarboxylic acid, di-C8-10-alkyl esters		
- 1,2-benzenedicarboxylic acid, di-C16-18-alkyl esters		
10,000 – 100,000 tonnes per year		
- DMP		
- 1,2-benzenedicarboxylic acid, benzyl C7-9-branched and linear alkyl esters		
100,000 – 1,000,000 tonnes per year		
- DEHP	X (since 28/10/2008)	X (sunset date: 21/02/2015) [a]
- DiNP		
- Bis(2-propylheptyl) phthalate		
- 1,2-benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich		
- 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich		
Intermediate use only		
- 1,2-benzenedicarboxylic acid, di-C1-13 alkyl esters		

[a] Except for the use in the immediate packaging of medicinal products.

Member states or ECHA can also decide to restrict a chemical's use or to make it subject to a prior authorisation (ECHA, 2014). Regarding phthalates (see also Section I.5.3), restrictions have been applied for the use of DEHP, DnBP, BBP, DiNP, DiDP and DnOP in toys and childcare articles (Official Journal of the European Union, 2006b).

I.5.2 Water Framework Directive

The European Water Framework Directive (WFD) is the short name of Directive 2000/60/EC of the European Parliament and the Council, establishing a framework for the Community action in the field of water policy. This Directive was published on December 22nd, 2000 and entered directly into force. It requires that all inland and coastal waters achieve a good qualitative and quantitative ecological status by 2015. According to Article 16 of the WFD, environmental quality standards (EQSs) should be developed for pollutants that present a significant risk to water and/or the aquatic biosphere. Furthermore, these pollutants should be identified by the European Commission and classified as priority substances (Official Journal of the European Union, 2000).

In Directive 2008/105/EC, EQS limits were established for 33 priority substances in surface waters. This Directive also stated that Member States should monitor sediment and biota, as appropriate, at an adequate frequency to provide sufficient data for a reliable long-term trend analysis of those priority substances that tend to accumulate in sediment and/or biota. Regarding phthalates, EQS limits have only been imposed for DEHP. In both inland and other surface waters, the annual average EQS limit for DEHP amounts 1.3 µg/l (Official Journal of the European Union, 2008).

In Flanders (Belgium), the conclusions of the WFD and Directive 2008/105/EC are implemented in the Flemish Environmental Permitting Regulation, also known as the "VLAREM" regulation (LNE, 2014). The Flemish Environment Agency (VMM) is responsible for the monitoring of DEHP (and other phthalates) in surface waters. For this purpose, surface water samples of various locations in Flanders are collected bimonthly (VMM, 2011). In Table 8, an overview is given of the analytical results of phthalates in Flemish surface water samples for monitoring year 2010.

Table 8: Phthalate levels (in µg/l) in Flemish surface water samples in 2010 (VMM, 2011).

Compound	No. of detected samples	Min.	P10	P50	P75	P90	P99	Max.
DMP	4/293	ND	ND	ND	ND	ND	<0.4	0.6
DEP	27/292	ND	ND	ND	ND	ND	0.6	2.1
DnBP	21/289	ND	ND	ND	ND	ND	0.5	0.8
BBP	20/293	ND	ND	ND	ND	ND	0.3	0.7
DPP	0/293	ND	ND	ND	ND	ND	ND	ND
DCHP	1/293	ND	ND	ND	ND	ND	ND	<0.4
DEHP	151/293	ND	ND	<0.2	0.3	0.4	5.6	10

DPP: di-*n*-pentyl phthalate; ND: not detected; Limits of detection (LODs): 0.1 µg/l (BBP, DPP and DEHP) and 0.2 µg/l (DMP, DEP, DnBP and DCHP).

I.5.3 Toys and childcare articles

As already mentioned in Section I.5.1, the use of certain phthalates for the production of toys and childcare articles is restricted. Since January 16th, Directive 2005/84/EC and the REACH regulation have banned the use of DEHP, DnBP and BBP – as substances or in mixtures – in all kind of toys and childcare articles in concentrations above 0.1% by weight of the plasticised material in Europe. For the purpose of this legislation, *childcare article* means any article intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children. Furthermore, the use of DiNP, DiDP and DnOP – as substances or in mixtures – is prohibited in toys and childcare articles that can be placed in the mouth by children, in concentrations above 0.1% by weight of the plasticised material (Official Journal of the European Union, 2005; 2006b). All Member States have

been obliged to implement Directive 2005/84/EC in national regulations. For Belgium, restrictions about the use of DEHP, DnBP, BBP, DiNP, DiDP and DnOP in toys and childcare articles are published in the Royal Decree of July 6th, 2006 (Belgisch Staatsblad, 2006).

I.5.4 Cosmetic and personal care products

In 2007, the Scientific Committee on Consumer Products (SCCP) of the European Commission published its opinion on the use of phthalates in cosmetic products (SCCP, 2007). In their report, they refer to, among others, the Cosmetics Directive 76/768/EC (Official Journal of the European Union, 1976) which states that the use of substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR) is prohibited in cosmetic products. This means that, with regard to phthalates, the use of DEHP, DnBP and BBP in cosmetic and personal care products is not authorised in the European Union. Phthalates like DEP are not regulated, which means that these compounds may be used in cosmetics available on the European market.

I.5.5 Medical devices

General regulations and requirements for medical devices have been set out in the Medical Devices Directive 93/42/EEC (Official Journal of the European Union, 1993b). According to this Directive, medical devices may only be placed on the market and put into service if they meet the requirements for safety and performance set out in the Directive.

Currently, there are no restrictions or bans in the European Union regarding the use of phthalates in medical devices notwithstanding the fact that some phthalates can cause adverse health effects in humans and/or that they are classified as CMR substances. Some European Member States, like Denmark, are urging the European Union to include phthalates on the agenda during negotiations on new rules for medical devices and to work on a reduction of phthalates in medical devices (Danish EPA, 2013).

I.5.6 Food contact materials

There are numerous existing regulations at both European and national levels regarding the correct use of materials and articles intended to come into contact with food. First, there are the Framework Regulation 1935/2004 (Official Journal of the European Union, 2004) and the Good Manufacturing Practice Regulation (Official Journal of the European Union, 2006c), which are both general regulations set up to secure a high level of protection of human health and the interest of consumers.

Next to these general legislations, there are regulations specific to one type of food contact material. For instance, Commission Regulation (EU) No 10/2011 declares specific rules for plastic materials (Official Journal of the European Union, 2011b). In this Regulation, it is stated that plastic materials and articles may not transfer their constituents to foodstuffs in quantities exceeding 10 mg/dm² of surface area of the plastic material or 60 mg/kg of foodstuff. This overall migration limit is a measure of the inertness of the material and prevents an unacceptable change in the composition of foodstuffs. As another example, Annex I of Regulation 10/2011 contains the “Union” list of substances (i.e. authorised monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids) that are approved to be intentionally used in the manufacture of plastic layers in plastic materials and articles. For every substance

mentioned in this list, restrictions and/or specific migration limits (SMLs) are declared. SMLs are generally based on available toxicological data or on exposure limit values (see also Section I.5.7) established by the European Scientific Committee on Food or the European Food Safety Authority (EFSA). To set these SMLs, it is assumed that every day during lifetime, a person of 60 kg consumes 1 kg of food packed in plastic always containing the substances at the maximum permitted quantity (European Commission, 2012). Table 9 gives an overview of the restrictions and SMLs that were set up for phthalates.

The testing of substance migration from plastic materials into foodstuffs is described in Annex V of Regulation 10/2011 (Official Journal of the European Union, 2011b). Basically, migration tests imply that a plastic material is brought into contact with a food product for a certain time and at a certain temperature. The time and temperature of contact have to replicate realistic conditions as much as possible and are specified in Annex V of Regulation 10/2011 as well. Because it is not always possible to use foodstuffs for testing food contact materials, food simulants are introduced to replace food products during migration testing. Depending on the food type to be simulated, different food simulants can be used (Table 10). The use of food simulants for compliance testing is handled in Annex III of Regulation 10/2011.

Table 9: Restrictions and specific migration limits for phthalates to be used in plastic food contact materials.

Compound	SML	Restrictions
DnBP	0.3 mg/kg	To be used only as: (a) plasticiser in repeated use materials and articles contacting non-fatty foods; (b) technical support agent in poly-olefins in concentrations up to 0.05 % in the final product.
BBP	30 mg/kg	To be used only as: (a) plasticiser in repeated use materials and articles; (b) plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC; (c) technical support agent in concentrations up to 0.1% in the final product.
DEHP	1.5 mg/kg	To be used only as: (a) plasticiser in repeated use materials and articles contacting non-fatty foods; (b) technical support agent in concentrations up to 0.1% in the final product.
DiNP + DiDP	9 mg/kg	To be used only as: (a) plasticiser in repeated use materials and articles; (b) plasticiser in single-use materials and articles contacting non-fatty foods except for infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC; (c) technical support agent in concentrations up to 0.1% in the final product.

In practice, various mixtures of food types are possible, for instance foods that are both acidic and fatty like yoghurt. Table 2 of Annex III of Regulation 10/2011 (Official Journal of the European Union, 2011b) gives an overview of which simulant should be used for which type of foodstuff. For some fatty food products, the result obtained in migration tests with simulant D2 is higher than that obtained in migration tests with the foodstuff itself. Therefore, the result should be corrected by applying a “reduction factor” appropriate to the particular situation. These fat reduction factors can also be found in Table 2 of Annex III.

Table 10: Overview of food simulants that can be used during migration testing.

Food simulant	Food type
Simulant A = ethanol 10% (v/v)	Aqueous foods (i.e. aqueous foods with a pH >4.5)
Simulant B = acetic acid 3% (w/v)	Acidic foods (i.e. aqueous foods with a pH ≤ 4.5)
Simulant C = ethanol 20% (v/v)	Alcoholic foods with an alcohol content of up to 20% and foods containing a relevant amount of organic ingredients that render the food more lipophilic
Simulant D1 = ethanol 50% (v/v)	Alcoholic foods with an alcoholic content of above 20% and oil-in-water emulsions
Simulant D2 = vegetable oil	Foods containing free fats at the surface
Simulant E = poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Dry foods

I.5.7 Exposure limit values

To assess risks associated with phthalate exposure, estimated phthalate intake values can be compared with exposure limit values. These values are estimates of the daily intake of a chemical which can occur over a lifetime without appreciable risk for human health and are established by authorities like EFSA, the WHO or the American Environmental Protection Agency (US EPA). Examples of exposure limit values are tolerable daily intake (TDI) values and reference dose (RfD) values (WHO, 2000a).

Since there are, in the majority of cases, inadequate data from humans to permit calculation of TDI or RfD values, exposure limit values are mostly based on end-points observed in animal studies. In such toxicological experiments, animals are exposed to chemical substances at certain dose ranges. After the experiment, a dose-response relationship is made and the “no observed adverse effect level” (NOAEL) is determined. The NOAEL is the highest dose of a substance that causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism. By taking into account a safety or uncertainty factor, NOAELs are extrapolated to exposure limit values applicable to humans. When a NOAEL cannot be determined, the “lowest observed adverse effect level” or LOAEL may be used to derive exposure limit values for humans. To obtain correct exposure limit values, it is essential that this type of values are based on end-points observed in animals that are also relevant to the toxicity of humans (Nordberg et al., 2004; WHO, 2000a). In Table 11, an overview is given of the TDI and RfD values that were established by EFSA (2005a; 2005b; 2005c; 2005d; 2005e), WHO (2003) and by US EPA (2007) for selected phthalate compounds.

Table 11: TDI and RfD values established for some phthalate compounds.

Compound	TDI (mg/kg bw/day)	RfD a (mg/kg bw/day)
DEP	5 (WHO, 2003)	0.8 (US EPA, 2007)
DnBP	0.01 (EFSA, 2005a)	0.1 (US EPA, 2007)
BBP	0.5 (EFSA, 2005b)	0.2 (US EPA, 2007)
DEHP	0.05 (EFSA, 2005c)	0.02 (US EPA, 2007)
DiNP	0.15 (EFSA, 2005d)	-
DiDP	0.15 (EFSA, 2005e)	-

[a] Reference dose for chronic oral exposure.

I.6 Methods to assess human exposure to phthalates

The assessment of human exposure to chemicals can follow on two main approaches: measuring or modelling. While these two methods can be considered to be complementary, they often also depend on each other. For example, measured concentrations are often required as inputs to exposure models and are essential to validate model results, whereas modelling is often needed to calculate and gain insight in a population's exposure starting from measured concentrations in different environmental media. In the context of health impact assessment, modelling can be applied to produce results applicable to past, future or other hypothetical scenarios (IEHIAS, 2014; WHO, 2005). In the next sections, both approaches for assessing human exposure will be discussed in detail. A further distinction is made between the assessment of the total, integral exposure via the various common exposure routes and exposure exclusively via the diet.

I.6.1 Measuring human exposure to phthalates

I.6.1.1 Measuring integral exposure

Integral human exposure to phthalates can be measured in two ways: directly or indirectly. Direct exposure assessments rely on biomonitoring studies in human populations. In this type of approach, the internal exposure (i.e. the body burden) is calculated by measuring biomarkers (chemicals, their metabolites and/or specific reaction products) in human specimens like urine, blood, and so on (Clark et al., 2011; Wittassek et al., 2011). With respect to the calculation of the body burden of phthalates in humans, phthalate metabolites are usually measured in urine and from the measured metabolite concentrations, the exposure to the parent compound is derived. To do this, the following model by David (2000) is often used:

Equation 1

$$DI_{direct} = \frac{UE \times CE}{1000 \times (f_{UE}/100)} \times \frac{MM_d}{MM_m}$$

With:

- *DI*: the daily internal intake of the phthalate diester (in µg kg^{-1} bw day^{-1});
- *UE:* the creatinine-corrected urinary metabolite concentration (in µg g^{-1});
- *CE*: the creatinine clearance rate normalised by body weight (in mg kg^{-1} bw day^{-1});
- f_{UE}: the molar conversion factor relating urinary excretion of metabolite to diester (in %);
- MM_d: the molecular mass of the diester (in g mol^{-1});
- MM_m: the molecular mass of the monoester (in g mol^{-1}).

In this equation, the value for the molar conversion factor, f_{UE}, is crucial for a correct estimation of the exposure to the parent compound. Various volunteer studies are necessary to determine f_{UE} values that are as accurate as possible (Clark et al., 2011). In Table 12, an overview is given of f_{UE} values available for the phthalate compounds considered in this PhD dissertation (Clark et al., 2011; Wittassek et al., 2011).

Values for the creatine clearance rate, *CE,* vary substantially among individuals because of various genetic and biologic factors like gender, age and muscle mass (Barr et al., 2005). For instance, Kohn

et al. (2000) assumed *CE* values of 23 and 18 mg kg^{-1} bw day^{-1} for male and female adults respectively, and in the study of Calafat and McKee (2006), *CE* values of 20, 11 and 9.8 mg kg^{-1} bw day^{-1} were adopted for adults combined, children and infants, respectively.

To date, numerous researchers have assessed the integral human exposure to phthalates relying on the human biomonitoring approach described above. Examples are, among others, Becker et al. (2004), CDC (2009), Geens et al. (2014) and Lee et al. (2014).

Table 12: Overview of available molar excretion factors of phthalate metabolites in urine.

Parent compound	Metabolite	f_{UE} (%)	Reference
DMP	MMP	69	(Clark et al., 2011)
DEP	MEP	69	(Clark et al., 2011)
DiBP	MiBP	69	(Clark et al., 2011)
DnBP	MnBP	69	(Clark et al., 2011; Wittassek et al., 2011)
BBP	MBzP	73	(Clark et al., 2011; Wittassek et al., 2011)
DEHP	MEHP	5.9-12	(Clark et al., 2011; Wittassek et al., 2011)
	5OH-MEHP	23	(Clark et al., 2011; Wittassek et al., 2011)
	5oxo-MEHP	15	(Clark et al., 2011; Wittassek et al., 2011)
	5cx-MEPP	19	(Clark et al., 2011; Wittassek et al., 2011)

Indirect studies, on the other hand, measure contaminant concentrations in various exposure media (e.g. air, water, food, dust, soil and consumer products) and combine these with survey or questionnaire data on personal lifestyle, product use and food consumption in order to calculate daily exposure through these pathways (Clark et al., 2011; Wittassek et al., 2011). Combining these external exposure estimates with organ- and situation-specific uptake rates, the daily internal exposure can be calculated according to the following equation derived by Clark et al. (2011):

Equation 2

$$DI_{indirect} = \sum \left(C_i \times IR_i \times \frac{A_i}{BW} \right)$$

With:

DI: the daily internal contaminant intake (in µg kg^{-1} bw day^{-1});
C_i: the contaminant concentration in medium *i* (in µg g^{-1});
IR_i: the intake rate of medium *i* (in g day^{-1});
A_i: the absorption factor of medium *i* (-);
BW: the body weight (in kg bw).

Several research groups have calculated human exposure to phthalates using the indirect approach (for instance, Clark et al., 2011; Lee et al., 2014; Wormuth et al., 2006). According to Franco (2007), the monitoring data used in this approach should be recent enough and of high quality in order to be able to correctly estimate daily human exposure to phthalates.

The major advantage of human biomonitoring studies (i.e. the direct approach) over indirect studies is that biomarker studies using phthalate metabolites are less subject to concerns over sample contamination, since phthalate metabolites are far less likely to arise from sample contamination

than their parent compounds. However, in contrast with indirect studies, human biomarker studies do not provide information on the source and route of exposure (dermal, oral and/or inhalation); they represent a single cumulative measure of exposure combining multiple sources and routes (Clark et al., 2011; Wittassek et al., 2011).

I.6.1.2 Measuring dietary exposure

To estimate the specific human exposure via dietary intake, contaminant concentrations in foods (C_{food}; in mg kg^{-1}) are multiplied with their corresponding intake rates (q_{food}; in kg day^{-1}). The sum of these individual food contaminant intake values is corrected for body weight (BW; in kg bw) in order to obtain the daily contaminant exposure via the diet (E_{diet}; in mg kg^{-1} bw day^{-1}) (Lambe, 2002). Equation 3 summarises these relations:

Equation 3

$$E_{diet} = \frac{\sum_{i=1}^{n}(C_{food} \times q_{food})}{BW}$$

Depending on the level of refinement of the dietary intake assessment, five different methods can be discerned. The following paragraphs describe these methods in increasing order of refinement. To conduct all these methods, simple (i.e. deterministic) or complex (i.e. probabilistic) approaches can be considered (see also Section I.6.2.3).

a) The per capita method

The *per capita* method uses food balance sheets to obtain per capita consumption intake rates. These rates are then multiplied with residue levels (permitted or analysed) of chemicals in individual food products.

b) Total diet or market basket study

In *total diet* or *market basket* studies, the dietary intake is estimated for a specific population group. Based on individual food consumption data, a list of food products is established, covering at least 90% of a typical diet consumed by the population under consideration. Before analysis, the selected foods are prepared according to standard household procedures and related products are separated into groups. Depending on the number of food groups, a distinction is made between total diet studies used for screening and total diet studies used for refined dietary exposure assessments. When used for screening, total diet studies are based on a limited number of food group samples (usually 20 to 30) to represent the whole diet. For refined dietary exposure assessments, the grouping of food products occurs at a much more refined level: food products are pooled into 200 to 300 different food group samples in order to be able to identify more clearly the food groups contributing most to dietary exposure. Multiplying the contaminant group concentrations with consumption estimates results in an estimation of the overall dietary intake (EFSA et al., 2011; Parmar et al., 1997). Total diet studies have been used by, among others, Petersen and Breindahl (2000) and Bradley et al. (2013b) to estimate human dietary exposure to phthalates.

c) The model diet method

The *model diet* method estimates the consumption of non-average individuals by defining hypothetical diets in which consumption values may be exaggerated in a *worst case* approach. This method is intended to formulate conclusions and policy advices aiming to protect the majority of the population. Predicted consumption values then are combined with residue concentrations to estimate dietary exposure.

d) Duplicate diet studies

Duplicate diet studies are used to assess the actual dietary exposure of a particular at-risk group. For the duration of the study, all foods consumed by an individual are collected in duplicate, with the duplicates being homogenised and analysed for the contaminant of interest. Intake is then estimated by multiplying the daily amount of food consumed by the contaminant concentration detected in the duplicate portions provided (Parmar et al., 1997). Specifically for dietary exposure to phthalates, duplicate diet studies have been used by Fromme et al. (2007b) and Tsumura et al. (2001a; 2003), among others.

e) The dietary survey method

Lastly, the *dietary survey* method determines dietary exposure to chemicals for the general population or a subgroup thereof by combining food consumption data derived from dietary surveys, with residue data derived from monitoring or surveillance programs. The food consumption data used in this approach are usually collected for a representative sample of the population using weighted diary surveys, 24h recalls or food frequency methods (Parmar et al., 1997).

I.6.2 Modelling human exposure to phthalates

I.6.2.1 Classification of models

There are many different ways to classify exposure models. With regard to the objectives of this PhD dissertation, three model characteristics are important and will be further discussed in the following paragraphs: (1) mechanistic versus empirical models, (2) deterministic versus probabilistic models and (3) dynamic versus static models (Dilks and Pendergast, 2000; Whitman et al., 1997; WHO, 2005).

a) Mechanistic versus empirical models

Mechanistic exposure models mostly make use of process, physico-chemical properties and/or mass relationships to predict exposure. Such models are mathematical constructs (i.e. they are represented by equations) that attempt to simulate the actual physical and chemical reality relevant for the exposure of interest. Mechanistic exposure models can be applied in situations where no measured exposure data are available or where such data are impossible to monitor experimentally (WHO, 2005). Compared to empirical models, mechanistic models are considered to be more robust when used outside the range of conditions for which they are developed (IEHIAS, 2014). Mechanistic models can also give more insight into the detailed dynamics of the exposure process and can provide a solid scientific basis for formulating exposure measures or policy advice.

Empirical models are numerical representations of relationships between input and output variables based on historic measurements. These models are data-driven and there are no grounds other than experimental confirmation that determine whether an empirical model can be used to calculate exposure in other systems (locations or populations) or even in the same system at another time point (WHO, 2005). Therefore, empirical models should always be validated and possibly calibrated again when used outside the range of the original data set for which they were developed (IEHIAS, 2014).

b) Deterministic versus probabilistic models

Deterministic exposure models are models in which variables have fixed point values so that the system, at any time, is entirely defined by the initial and/or boundary conditions chosen (WHO, 2005). These models result in point estimates of chemical exposures in a population or subpopulation and thus do not provide any insight into the range of possible exposures that may occur within a population. Deterministic models are commonly used as a first step in assessing exposure because they are relatively simple and inexpensive to carry out (Lambe, 2002).

Probabilistic or stochastic exposure models are models that consider the stochastic nature of one or more input parameters or variables, by describing them using a statistical distribution rather than a single value. They allow exposure assessors to integrate in the model both the variability (true, inherent heterogeneity) and the uncertainty (lack of knowledge) that may exist in the exposure variables and thus to generate distributions of possible resulting exposures. Most probabilistic models are too complex to be solved analytically and approaches such as Monte Carlo simulation are often used to obtain the output distributions (Lambe, 2002; WHO, 2005). For policy makers, mechanistic-stochastic models are likely to be the most informative (IEHIAS, 2014).

Alternatively, semi-probabilistic (also called simple distribution or semi-distributional) exposure models can be used. In these models, fixed point values of contaminant concentrations are combined with distributions of consumption. The results of this approach are more informative than the deterministic approach, since the variability in consumption data is taken into account. However, semi-probabilistic exposure models still retain conservative assumptions related to contaminant levels and therefore can only be considered to give upper bound estimates of exposure (Lambe, 2002).

c) Dynamic versus static models

Dynamic models are designed to consider variations in factors of a system (e.g. an environmental compartment or a human) over time and are typically represented by differential equations (Dilks and Pendergast, 2000). In this manner, dynamic models can provide information about the state of a system at a given instance in time or can generate performance measures of a system over a given period of time (Whitman et al., 1997).

In contrast to dynamic models, static models provide predictions for only a single set of conditions (Dilks and Pendergast, 2000). For exposure modelling, often a critical or worst case set of conditions is selected in order to obtain conservative estimates. Static models attempt to provide static

representations of dynamic systems by describing systems that are in equilibrium. This way, static models are time-independent (Whitman et al., 1997).

I.6.2.2 Modelling integral exposure

Integral human exposure to chemicals can be modelled internally as well as externally. To model exposure in an *internal* way, physiologically based pharmacokinetic (PBPK) models are often used. These models calculate doses of chemicals and their metabolites over a wide range of exposure conditions in the human body. In PBPK modelling, the body is subdivided into a series of compartments that represent specific organs, or lumped tissue and organ groups, with appropriate volumes, blood flow rates, and pathways of metabolism. PBPK models need to be carefully calibrated to ensure that the results are consistent with toxicokinetic measurements, and validated against human biomonitoring data in order to disentangle the inter-individual biologic variability in toxicokinetic behaviour from the uncertainty in the model parameters and input data (IEHIAS, 2014). PBPK models can be either generic or chemical specific. Regarding phthalates, PBPK models have already been developed for DnBP in rats (Clewell et al., 2008) and for DEHP in humans (Lorber et al., 2010).

External exposure models generally combine concentration data on contact media (air, soil, food, dust, consumer products, et cetera) – either measured or modelled – with data on contact events, contact times and intake and absorption rates to produce exposure estimates for different routes of entry. There are integral exposure models that combine all routes of exposure (inhalation, dermal contact and dietary and non-dietary ingestion) as well as models for single routes of exposure (WHO, 2005). Franco et al. (2007), for instance, used the EUSES (European Union System for the Evaluation of Substances) model to predict human exposure to DnBP and DEHP via ingestion and inhalation. EUSES is a multimedia fate and exposure model developed by Vermeire et al. (2005; 1997) and is commissioned by the European Union as the preferred modelling tool for chemical risk assessments (ECHA, 2012). As the EUSES model does not consider exposure via consumer products, Müller et al. (2003) used the EUSES model in combination with the CONSEXPO (*CONSumer EXPOsure*) model (van Veen, 2001) to predict integral exposure to DEHP, DnBP, BBP, DiDP and DiNP in the Danish population.

I.6.2.3 Modelling dietary exposure

A dietary exposure model, in essence, calculates contaminant intakes as the product of the amounts of food items consumed during the duration of the exposure of interest and the contaminant concentrations in those food items (Lambe, 2002; WHO, 2005). *Deterministic* dietary exposure models result in point estimates of the intake of food chemicals in a population. For this type of approach, average contaminant concentrations in foods are usually combined with average consumption rates of a population or subpopulation. *Probabilistic* dietary exposure models provide the advantage of describing the probability for varying levels of intake and concentration to occur. Most of the time, however, *semi-probabilistic* models are used to calculate dietary exposure. These models combine deterministic and probabilistic approaches by employing distributions of food intake rates and fixed values for the concentrations (Lambe, 2002).

To accurately estimate dietary exposure to chemicals, various tools have been developed. Two examples of such tools are the “Monte Carlo Risk Assessment” software (MCRA) developed by the

Dutch National Institute of Public Health and the Environment (https://mcra.rivm.nl) and the American “National Cancer Institute” (NCI) method (Kipnis et al., 2009; Parsons et al., 2009; Tooze et al., 2010).

I.7 Outline and objectives

This PhD dissertation aims to estimate and evaluate the dietary exposure of the Belgian adult population to eight phthalate compounds: DMP, DEP, DiBP, DnBP, BBP, DCHP, DEHP and DnOP. In order to achieve this goal, specific objectives have been determined:

1. Obtain accurate and high quality data of phthalates in a wide range of commonly consumed food products and commonly used packaging materials available on the Belgian market;
2. Investigate contamination pathways that may contribute to the transfer of phthalates from the environment and from food contact materials into food products present on the Belgian market;
3. Develop a model that is able to calculate the environmental transfer of phthalates into Belgian agricultural products (animal feed, fruit, vegetables, grains, potatoes, meat, milk, eggs, liver, and so on);
4. Extend the developed model in order to calculate the influence of food packaging and food processing on phthalate levels in Belgian food items;
5. Validate the developed model by comparing predicted phthalate levels in Belgian agricultural products with analysed levels;
6. Link both analysed and modelled phthalate levels in foods to Belgian food consumption data;
7. Assess the dietary exposure of the Belgian adult population to phthalates based on both analysed and modelled phthalate concentration levels in foods;
8. Evaluate the overall health risks of dietary phthalate exposure against exposure limit values for the Belgian adult population.

A schematic overview of these specific objectives is presented in Figure 3.

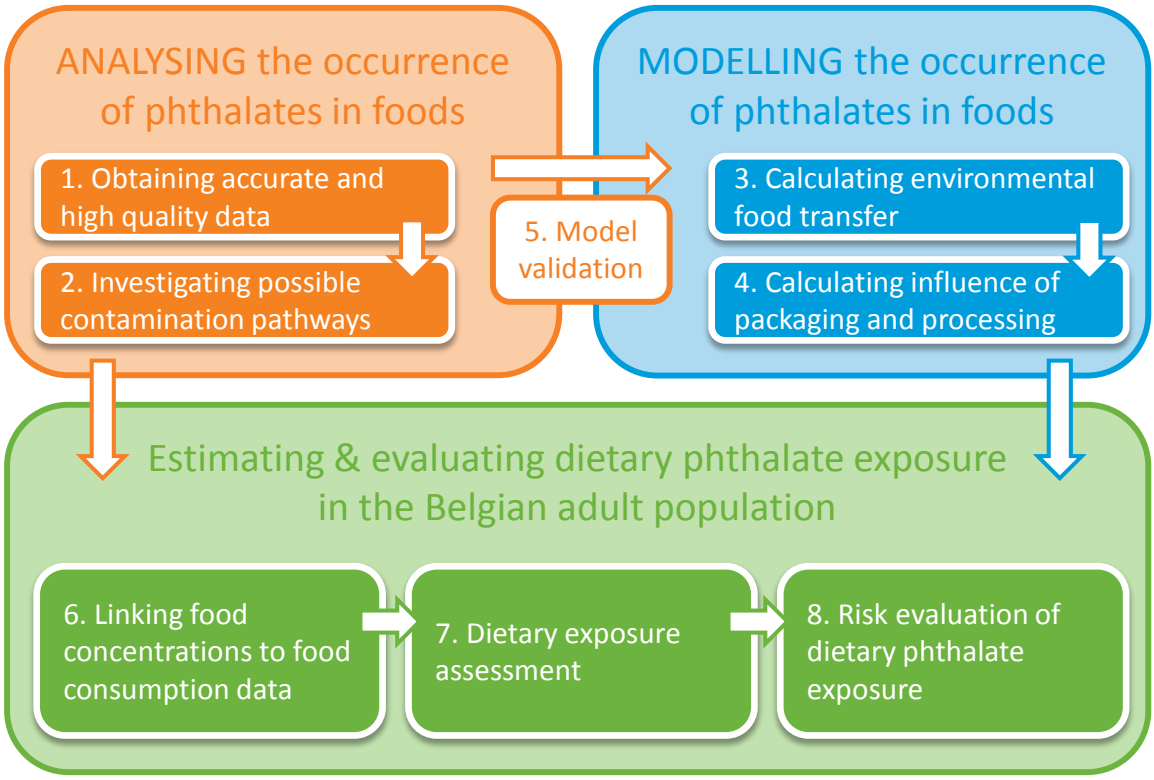

Figure 3: Schematic presentation of the objectives of this PhD dissertation.

The research carried out in this doctoral project will be presented in three main parts.

In the first part (containing six chapters), studies in which phthalate levels are analysed in Belgian food products or in which analysed food concentrations are used in order to assess human dietary exposure to phthalates are provided. This part focuses on objectives 1, 2, 6, 7 and 8 of this thesis (Figure 3). The first three chapters discuss the results obtained in the framework of the "PHTAL" project. This study was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (Contract No. RT/08/1 PHTAL) and performed by a consortium of the Flemish Institute for Technological Research (VITO) and Ghent University (Department of Public Health). The next two chapters describe the results of a study in which phthalate contamination was investigated in a contemporary Belgian milk production chain. Finally, the last chapter of this part addresses the issue whether the occurrence of phthalates in foods is influenced by home-cooking.

The second part (containing two chapters) describes the work on modelling phthalate levels in foods or modelling human dietary exposure to phthalates. The first chapter describes the development and validation of the "EN-forc" model (i.e. ENvironmental Food transfer model for ORganic Contaminants), which meets objectives 3 and 5 of this dissertation (Figure 3). In the second chapter, the EN-forc model is extended to include the influence of food packaging and food processing on the occurrence of phthalates in food products, as well as the calculation of human dietary exposure to phthalates. In this way, objectives 4 to 8 of this thesis are fulfilled (Figure 3).

The different chapters of these two parts are independent papers, which have been published earlier in international peer-reviewed scientific journals.

The third part is a general discussion of the studies carried out within this doctoral project. In the first chapter, the most important research results and main discussion points obtained during this PhD project are summarised. In the subsequent chapters, the relevance for public health, possible suggestions for policy development and recommendations for further research are highlighted. The general discussion finishes with some concluding remarks.

II. Measuring phthalates in foods and their related exposure in the Belgian adult population

This part reports on the results of the three studies that were conducted during this PhD project in order to measure the occurrence of phthalates in foods and their related exposure in the Belgian adult population: 1) the PHTAL project, 2) a milk chain survey and a 3) heat treatment investigation.

The first three chapters discuss the results of the three objectives that were achieved during the Belgian PHTAL project, namely (1) to obtain accurate and sensitive data of phthalates in all kinds of food products and packaging materials sold on the Belgian market, (2) to gain a clear understanding of possible contamination pathways for phthalates in the Belgian food chain and (3) to estimate dietary exposure to phthalates in the Belgian population in a probabilistic way using the dietary survey method (see Section I.6.1.2). A schematic overview of these objectives and corresponding work packages is depicted in Figure 4. The PHTAL project was conducted between January 2009 and December 2011, funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (Contract No. RT/08/1 PHTAL) and performed by a consortium of the Flemish Institute for Technological Research (VITO) and Ghent University (Department of Public Health).

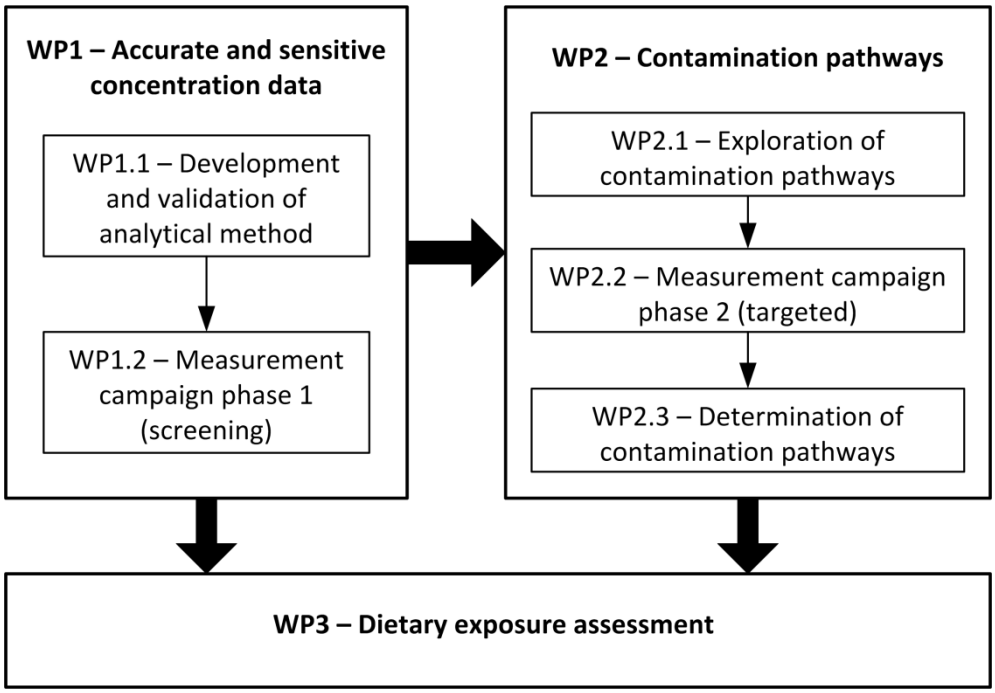

Figure 4: Schematic overview of the different work packages of the PHTAL project.

The next two chapters describe the results of the study in which phthalate contamination was investigated in a contemporary Belgian milk chain. The first chapter focuses on the occurrence of phthalates in Belgian raw cow's milk and thus on phthalate contamination at farm level. In the second chapter, phthalate contamination during the production process of milk powder (i.e. at industry level) and in several purchased milk and dairy product samples (i.e. at retail level) is described.

The last chapter of this part addresses the issue whether the occurrence of phthalates in foods is influenced by home-cooking. In this chapter, results are given of phthalate concentrations in both raw/unprepared and prepared starchy products (potato, rice and pasta), vegetables (carrot,

cauliflower, onion and paprika), meat (minced meat and pork chop) and fish (salmon). Heat treatments that were considered in this study are: boiling, steaming, frying in a frying pan with margarine, frying in a non-stick frying pan without margarine, deep-frying, grilling in oven without aluminium foil and grilling in oven with aluminium foil (i.e. “en papillote”).

II.1 PHTAL 1 – Analysis of phthalates in Belgian food products and packaging materials

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I. and Vanermen, G. (2012). ***Analysis of phthalates in food products and packaging materials sold on the Belgian market.*** *Food and Chemical Toxicology, 50, 2575-2583.*

Abstract

Phthalates are organic lipophilic compounds that are principally used as plasticiser to increase the flexibility of plastic polymers. Other applications are a.o. the use of phthalates in printing inks and lacquers. Human exposure to phthalates mainly occurs via food ingestion and can induce adverse health effects. In this study, the presence of eight phthalate compounds – dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP) – was investigated in 400 food products, divided over eleven groups, and packages sold on the Belgian market. For this purpose, suitable extraction techniques were developed and validated for four different matrices, namely high-fat foods, low-fat food products, aqueous-based beverages and packaging materials. The instrumental analysis was performed by means of gas chromatography-low resolution-mass spectrometry with electron impact ionisation (GC-EI-MS). A wide variety of phthalate concentrations was observed in the different groups. DEHP was found in the highest concentration in almost every group. Moreover, DEHP was the most abundant phthalate compound, followed by DiBP, DnBP and BBP. This survey is part of the PHTAL project, which is the first project that discusses phthalate contamination on the Belgian food market.

II.1.1 Introduction

Diesters of ortho-phthalic acid – hereafter referred to as “phthalates” – are organic lipophilic compounds, which are principally used as plasticiser to increase the flexibility of plastics such as polyvinyl chloride. Other applications are a.o. the use of phthalates in printing inks and lacquers, to which they are added to improve surface adhesion, flexibility and wrinkle resistance. Each year, about one million tonnes of phthalates are produced in Western Europe, of which di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) are the most dominant ones (Cao, 2010; Castle et al., 1989; CDC, 2009; ECPI, 2010; Stanley et al., 2003).

Phthalates and their metabolites have been reported to cause detrimental effects to human health. For instance, researchers demonstrated that di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), DEHP and DiNP can adversely affect the male reproductive system (Foster et al., 2000; Gray et al., 2000; Swan et al., 2005). Duty et al. (2003a) found an association between DNA damage in sperm and exposure to diethyl phthalate (DEP). Furthermore, Latini et al. (2003; 2004) revealed that DEHP can disrupt the human endocrine system and can induce premature delivery in humans. Bornehag et al. (2004) related high concentrations of DEHP in house dust with increased incidences of asthma and high BBP levels with increased rates of rhinitis and eczema in children. In Europe, risk assessment reports have been accomplished for DnBP, DEHP, BBP, DiDP and DiNP by the European Chemicals Bureau (ECB, 2003a; 2003b; 2004; 2007; 2008). Tolerable daily intakes (TDIs) have been specified by the European Food Safety Authority (EFSA) for these phthalates, namely 0.01 mg/kg bw for DnBP (EFSA, 2005a), 0.05 mg/kg bw for DEHP (EFSA, 2005c), 0.50 mg/kg bw for BBP (EFSA, 2005b) and 0.15 mg/kg bw for DiDP and DiNP (EFSA, 2005d; 2005e).

For humans, food intake is the most important exposure pathway for many phthalates, followed by ingestion of dust and inhalation of indoor air (Clark et al., 2003a; Fromme et al., 2007b; Rudel et al., 2003; Wormuth et al., 2006). During the last 30 years, numerous studies have been published concerning the presence of phthalate compounds in food and packaging materials. Studies included the analysis of phthalates in samples from duplicate diet studies[3] or from total diet studies[4] (Bopp and Altkofer, 2009; COT, 2011; Fromme et al., 2007b; MAFF UK, 1996; Petersen and Breindahl, 2000; Tsumura et al., 2001a; 2003) as well as in retail foodstuffs (Cocchieri, 1986; Jarosova, 2006; Peters, 2006; Pfannhauser et al., 1995; Pfördt, 2004; Poças et al., 2010; Tsumura et al., 2002b; Zhang et al., 2008) or both (Page and Lacroix, 1995). Bosnir et al. (2007) considered the migration of six phthalates – dimethyl phthalate (DMP), DEP, DnBP, BBP, DEHP and di-*n*-octyl phthalate (DnOP) – in soft drinks and mineral waters packed in polyethylene terephthalate. Other studies focused on the occurrence of DEP, DnBP, BBP, DEHP, DnOP and DiNP in ready-to-eat meals (Tsumura et al., 2001b; 2001c). Data on phthalates in all kinds of packaging materials – i.e. paper, cardboard, plastic, metal closures of glass jars, cans, etc. – have been reported in literature as well (Balafas et al., 1999; Bononi and Tateo, 2009; Fankhauser-Noti et al., 2006; Jarosova, 2006; Lopez-Espinosa et al., 2007; MAFF UK, 1995; Nerin et al., 1993; Page and Lacroix, 1995; Poças et al., 2010; Zhang et al., 2008).

Although many European researchers have investigated the occurrence of phthalate compounds in food and packaging materials, little information is available concerning the presence of phthalates in food products sold on the Belgian market and the intake of phthalates by the Belgian population. Therefore, by order of the Belgian Federal Public Service of Health, Food Chain Safety and Environment, the Flemish Institute for Technological Research (VITO) and Ghent University (Department of Public Health) conducted a research project (acronym: PHTAL) from January 2009 until December 2011 with the following main objectives:

- To obtain accurate and sensitive data of phthalates in all kinds of food products and packaging materials sold on the Belgian market;
- To gain a clear understanding of possible contamination pathways for phthalates in the Belgian food chain;
- To estimate dietary exposure to phthalates in the Belgian population.

This paper reports on the results of the measurement campaign that has been set up to achieve the first objective of the PHTAL project. Also the analytical procedure that was used to obtain these data is entirely discussed. The article focuses on the following eight phthalate compounds: DMP, DEP, diisobutyl phthalate (DiBP), DnBP, BBP, DEHP, dicyclohexyl phthalate (DCHP) and DnOP.

[3] A duplicate diet study is a study, in which test persons consume their ordinary diet, but for subsequent analysis, they prepare also a duplicate portion of all food products as prepared, served and consumed (Parmar et al., 1997).

[4] A total diet study or market basket is based on national food consumption surveys. All food products that are part of the national average diet are purchased and prepared as they would be consumed prior to analysis (Parmar et al., 1997).

II.1.2 Material and methods

II.1.2.1 Sample collection

Representative samples of widely consumed foods were purchased from various Belgian shops between May 2009 and July 2010. Sample selection was based on (1) consumption data from the most recent Belgian national food consumption survey (Devriese et al., 2006) and (2) the likelihood that foodstuffs contain phthalates (e.g. high-fat food products and foodstuffs packed in printed packaging materials) (Castle et al., 1989; Tsumura et al., 2002a). An overview of the selection is given in Table 13. In total, 400 samples (including twelve packaging materials) were analysed in this measurement campaign. Organically grown food products were investigated too, although their share in the total food consumption was relatively small. Brand name, packaging material and properties, fat content, shelf life, time and place of purchase, picture and – if relevant – product specific properties (e.g. pH, preserving agent) of the foods were stored in a database.

Table 13: Overview of the selected food products and packaging materials for the measurement campaign. The number of samples is given between brackets.

Group	Subgroup
Fruits and vegetables (27)	Fruits (7)
	Vegetables (17)
	Nuts (3)
Milk and dairy products (56)	Milk (8)
	Milk beverages (8)
	Cheese (21)
	Fresh cheese, yoghurt, cream, dessert, etc. (19)
Cereals and cereal products (47)	Bread (18)
	Breakfast cereals (7)
	Pasta (11)
	Rice (4)
	Flour, starches and oatmeal (7)
Meat and meat products (22)	Meat (13)
	Meat products (9)
Fish and fish products (18)	Fish (10)
	Fish products (6)
	Crustaceans (2)
Fat and oils (26)	Vegetable oils (15)
	Vegetable fat (8)
	Animal fat (3)
Snacks (28)	Salty biscuits (4)
	Sweet biscuits and cakes (10)
	Confectionery (4)
	Syrup, sugar, honey, popcorn, chocolate spread, etc. (10)
Condiments and sauces (40)	Condiments (7)
	Pesto (4)
	Mayonnaise (6)
	Mustard, vinaigrette, ketchup, curry, etc. (23)
Miscellaneous (22)	Ready-to-eat meals (22)
Baby food (17)	Milk powder (3)
	Fruit puree, vegetable puree, soup, etc. (14)
Beverages (85)	Beer (18)
	Soft drinks (25)
	Juices (22)
	Water (20)
Packaging materials (12)	Cardboard (5)
	Tetra brick (2)
	Plastic (5)

II.1.2.2 Reagents and materials

Dichloromethane, iso-propanol, acetone, *n*-hexane and sodium sulphate were purchased from Merck (Overijse, Belgium). DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP were supplied by Sigma-Aldrich (Bornem, Belgium). Standard solutions of these phthalate compounds were made in both dichloromethane (concentration 1 µg/ml) and iso-propanol (concentration 0.85 µg/ml). Deuterium-labelled phthalate compounds (d_4-DMP, d_4-DEP, d_4-DiBP, d_4-DnBP, d_4-BBP, d_4-DEHP and d_4-DnOP) were purchased from Sigma-Aldrich (Bornem, Belgium) as well and were used as internal standards in this study. Therefore, a standard solution of these compounds was prepared in dichloromethane at a concentration of 16 µg/ml. Calibration standard solutions of the native phthalate compounds (0.05, 0.1, 0.5 and 1.15 µg/ml) were prepared by serial dilution in dichloromethane. For obtaining calibration curves, 1 ml of every standard was spiked with 25 µl of the internal standard solution.

II.1.2.3 Control of blank concentrations

Due to the omnipresence of phthalates in the laboratory environment, sample contamination can occur in every stage of the analytical procedure. Therefore, control of the blank concentrations is a condition sine qua non to perform reliable and sensitive analyses of phthalates. Prior to analysis, solvents, reagents, laboratory glassware, the evaporation system, the gel permeation chromatography (GPC) system and the gas chromatography-mass spectrometry (GC-MS) system were tested for their contamination with phthalates. Based on these results, a list of special guidelines was set up to reduce the risk of contamination during sample preparation and analysis:

- The laboratory where sample preparation took place, was only allocated to the analysis of phthalates;
- Laboratory glassware and sodium sulphate were heated at 450 °C for at least four hours and were covered with aluminium foil prior to use;
- Before use, glassware, syringes, spatula, sodium sulphate, etc. were rinsed carefully with dichloromethane;
- Glass columns instead of disposable columns were used to dry food samples chemically with sodium sulphate;
- No laboratory gloves were used during sample preparation and analysis;
- For evaporation under nitrogen atmosphere, the needles were removed;
- One GPC and one GC-MS system were dedicated to the analysis of phthalates.

II.1.2.4 Sample preparation

Suitable extraction techniques were developed for the various food matrices and packaging materials. A distinction was made between high-fat foods (fat content of more than one percent on a fresh weight basis), low-fat products (fat content of less than one percent), aqueous-based beverages (beer, soft drinks, juices and water) and packaging materials (cardboard, tetra brick and plastic).

High-fat food products were first homogenised by shaking or stirring. In case of meat, fish and similar products, samples were cut into small pieces. After homogenisation, a representative amount of every sample was taken. If a sample contained a high amount of water, the sample was chemically dried with sodium sulphate. Then, a fat extraction with acetone/*n*-hexane (1:1) took place. For every 10 g of sample, 20 ml of extraction solvent was added. After being shaken on a shaking table for 30

min, the mixture was centrifuged and the supernatant was evaporated under nitrogen until constant weight. Of the total fat residue, 0.5 g was dissolved in 2 ml of dichloromethane and 25 µl of the internal standard solution was added. To remove interfering fats and other co-extracted compounds, a purification step was required. In this survey, cleanup was performed by gel permeation chromatography (GPC). The GPC system consisted of a HPLC pump (Shimadzu, LC-20AT), an autosampler (Shimadzu, SIL-20AC), a fraction collector (Shimadzu, FRC-10A) and a UV-VIS detector (Shimadzu, SPD-20A). Separation took place on a Waters Envirogel column (19 x 300 mm), which was packed with styrene divinylbenzene copolymer particles (pore size 100 Å). Dichloromethane acted as mobile phase (flow rate of 4 ml/min). The fraction between 15.0 and 18.5 min contained the compounds of interest and was collected automatically. The final extract was evaporated under nitrogen atmosphere to approximately 1 ml.

Prior to extraction, *low-fat foods* were also homogenised by means of shaking, stirring or cutting into pieces. A representative amount of the sample – i.e. 10 g – was weighed and, if the sample contained a high amount of water, chemically dried with sodium sulphate. Subsequently, 25 µl of the internal standard solution was added to the sample, weighed in a 60 ml-vial. The extraction was performed by adding 40 ml of acetone/*n*-hexane (1:1), followed by centrifugation during 10 min at 3000 rpm. The supernatant was evaporated under nitrogen atmosphere and the solvent was exchanged to 20 ml of dichloromethane. The final extract was brought to a volume of approximately 1 ml. If necessary, cleanup was performed by GPC.

For *aqueous-based beverages*, a liquid-liquid extraction was applied. After homogenisation, 500 ml of the sample, to which 25 µl of the internal standard solution was added, was brought into a separating funnel and was vigorously shaken. Consequently, a liquid-liquid extraction was performed with 30 ml of dichloromethane. In case the organic layer still contained water, the extract was chemically dried with sodium sulphate. No purification step was required. Finally, the extract was evaporated under nitrogen atmosphere to approximately 1 ml.

Packaging materials were cut into pieces of about 1 cm². Subsequently, a representative subsample of 5 cm² was extracted for 60 min with 40 ml of *n*-hexane in an ultrasonic bath. To the extract, 25 µl of the internal standard solution was added. Finally, a solvent exchange to 20 ml of dichloromethane took place and the extract was evaporated under nitrogen atmosphere to a volume of approximately 1 ml. No purification step was required.

II.1.2.5 Instrumental analysis and quantification

The instrumental analysis of phthalates was performed by gas chromatography-low resolution-mass spectrometry with electron impact ionisation (GC-EI-MS). For this purpose, a gas chromatograph coupled with a mass selective detector of Agilent Technologies was used (Agilent 5975C inert XL EI/CI MSD with Triple-Axis Detector). After the injection of 1 µl of the sample at 250 °C in splitless mode, phthalates were separated on a DB-XLB column (60 m length, 0.25 mm internal diameter, 0.25 µm film thickness) with a non-polar stationary phase. The temperature of the GC oven was preset as follows: 50 °C during 1 min; increase of temperature to 320 °C at 15 °C/min and held constant at 320 °C during 15 min.

The different phthalate compounds were detected in selected ion monitoring (SIM) mode. For each compound, a target and a qualifier ion were selected on the basis of the intensity of signals (Table

14). For all phthalates except DMP, the dominant product ion *m/z* 149 was used for quantification; for DMP, *m/z* 163 was used. As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compound in a sample had to be within 20% of that observed in a standard solution.

Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. This allows correcting for possible losses during extraction and/or cleanup, since the deuterium-labelled phthalates were added to the samples prior to extraction and thus undergo extraction, cleanup and analysis in the same way as the phthalate compounds of interest. The following deuterium-labelled internal standards were applied: d_4-DMP, d_4-DEP, d_4-DiBP, d_4-DnBP, d_4-BBP, d_4-DEHP and d_4-DnOP. The retention times on the DB-XLB column and the characteristic *m/z* values for these compounds are summarised in Table 14 as well.

Table 14: Retention times, target ions and qualifier ions for the phthalates of interest and their corresponding deuterium-labelled internal standards.

Phthalate compound	Corresponding internal standard	Retention time (min)	Target ion (*m/z*)	Qualifier ion (*m/z*)
DMP	d_4-DMP	13.4	163	194
DEP	d_4-DEP	14.6	149	177
DiBP	d_4-DiBP	16.4	149	223
DnBP	d_4-DnBP	17.1	149	223
BBP	d_4-BBP	19.8	149	206
DEHP	d_4-DEHP	20.6	149	167
DCHP	d_4-DEHP	20.9	149	167
DnOP	d_4-DnOP	22.0	149	279
Internal standards				
d_4-DMP		13.4	167	198
d_4-DEP		14.5	153	181
d_4-DiBP		16.4	153	227
d_4-DnBP		17.1	153	227
d_4-BBP		19.7	153	210
d_4-DEHP		20.6	153	171
d_4-DnOP		22.0	153	283

II.1.2.6 Quality assurance and quality control measures

Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (12 samples at the most) to reduce the risk of contamination. The composition of the procedural blank depended on the extraction and cleanup procedure that were used. For aqueous-based beverages, 30 ml of dichloromethane, to which 25 µl of the internal standard solution was added, was brought into a separating funnel, where after the analytical procedure was applied as described in Section II.1.2.4. For high-fat food products, 25 µl of the internal standard solution was added to 2 ml of dichloromethane, followed by GPC and evaporation of the extract to approximately 1 ml. In case of low-fat foods and packaging materials, 40 ml of acetone/*n*-hexane (1:1) and 40 ml of *n*-hexane, respectively, were spiked with 25 µl of the internal standard solution and were exchanged and evaporated to 1 ml of dichloromethane.

The reference sample used was also matrix dependent. For aqueous-based beverages, the phthalates of interest were added to tap water in a concentration of 0.05 µg/kg fresh weight. Sunflower oil, to which the phthalates were added in a concentration of 250 µg/kg fat, was used as a reference

sample for the analysis of high-fat food samples. Sunflower oil without addition of the native compounds was analysed as well. For the analysis of low-fat food products, phthalate compounds were added to one of the selected food products in a concentration of 15 µg/kg fresh weight (sample intake of 10 g). Lastly, one of the selected packaging samples was applied as a quality control sample during the analysis of packaging materials. Therefore, phthalates in a concentration of 30 ng/cm² were added to the extract of this sample. For the various reference samples, the same extraction and cleanup procedure was followed as described in Section II.1.2.4. The recovery of the phthalate compounds was calculated as the ratio between the experimentally observed concentration and the theoretical concentration. The evolution of the recovery in each reference sample was followed in a quality control chart in order to preserve a good quality of the analytical procedure.

II.1.2.7 Reporting of results

Phthalate concentrations are expressed in micrograms per kilogram fresh weight for foodstuffs and beverages (µg/kg fresh weight) and as nanograms per square centimetre for packaging materials (ng/cm^2). For high-fat food products, the initial concentration was expressed in µg/kg fat. This result was converted to µg/kg fresh weight using the fat content either mentioned on the packaging or determined experimentally.

As already mentioned, phthalates are omnipresent in the laboratory. Limits of quantification (LOQs) are thus strongly dependent on the feasible blank concentrations. Therefore, LOQ values were calculated based on the phthalate concentrations detected in the procedural blanks. The LOQ of each individual phthalate compound equalled the sum of the average blank concentration and six times the standard deviation of replicate procedural blank measurements under reproducibility conditions (each replicate determination was obtained from an independent extraction).

II.1.3 Results

II.1.3.1 Method performance

The developed extraction techniques and instrumental conditions were subjected to a validation study in order to determine method performance characteristics and to guarantee the quality of the analytical procedure.

Table 15 summarises the performance characteristics that were achieved for the different sample matrices. For the recovery experiments, phthalate compounds were added to sunflower oil, fruit puree (baby food) and tap water in a concentration of 100 µg/kg fat, about 15 µg/kg fresh weight and 0.05 µg/kg fresh weight, respectively. The spiked concentrations for every matrix were based on earlier reported concentrations for that type of matrix in literature (Page and Lacroix, 1995; Pfördt, 2004; Tsumura et al., 2002b) and, if necessary, modified according to the phthalate concentrations determined in the first samples of every matrix. Each sample for the recovery experiments was also measured without addition of phthalate compounds in order to determine the appropriate blank concentration. Recoveries varied between 88% and 104%. The measurements of every sample matrix were carried out under reproducibility conditions. The reproducibility, expressed as the relative standard deviation (RSD), is also listed in Table 15. The RSD for each phthalate compound in the different food matrices was lower than 13%, which indicates a good reproducibility of the analytical procedure (Official Journal of the European Union, 2002).

Table 15: Overview of the method performance characteristics obtained for every matrix: recovery percentages with corresponding relative standard deviations (RSDs) between brackets and limits of quantification (LOQs). LOQ values for high-fat and low-fat foods varied between (sub)groups. Therefore, average LOQ values are given with corresponding minimum and maximum values between brackets.

Phthalate	Recovery (RSD)				LOQ			
	High-fat[a] (%)	Low-fat[b] (%)	Aqueous[c] (%)	Packaging[d] (%)	High-fat (µg/kg fat)	Low-fat (µg/kg fw)	Aqueous (µg/kg fw)	Packaging (ng/cm²)
DMP	93 (5)	100 (3)	99 (3)	90 (5)	5 (1-10)	0.2 (0.1-0.2)	0.01	0.1
DEP	97 (11)	99 (3)	100 (5)	85 (12)	40 (10-60)	2.0 (0.2-6.0)	0.03	0.5
DiBP	94 (7)	96 (5)	104 (13)	82 (12)	15 (5-15)	2.0 (0.2-5.0)	0.03	1.0
DnBP	96 (10)	96 (2)	101 (10)	82 (10)	20 (5-20)	4.0 (0.1-5.0)	0.03	1.5
BBP	100 (8)	88 (7)	96 (4)	85 (8)	20 (3-45)	1.5 (0.1-1.5)	0.01	0.5
DEHP	98 (11)	102 (11)	101 (9)	99 (14)	145 (25-230)	8.0 (0.3-20)	0.03	0.5
DCHP	95 (10)	91 (1)	88 (7)	91 (7)	35 (2-40)	1.0 (0.1-1.5)	0.01	0.5
DnOP	94 (5)	96 (2)	96 (3)	90 (6)	25 (1-50)	0.5 (0.1-0.5)	0.01	0.5

[a] Spiked sunflower oil samples at a phthalate level of 100 µg/kg fat (n=20); [b] Spiked baby food samples at a phthalate level of about 15 µg/kg fresh weight (n=6); [c] Spiked tap water samples at a phthalate level of 0.05 µg/kg fresh weight (n=17); [d] Spiked extracts of packaging samples at a phthalate level of 30 ng/cm² (n=4).

For the determination of the performance characteristics for the analysis of packaging materials, four different types of material were selected: a plastic bag, a printed cardboard and two different types of foil. These packaging materials were cut into pieces of about 1 cm², of which a representative sample of 5 cm² was taken. Each sample was spiked with the phthalate compounds of interest in a concentration of 30 ng/cm². Afterwards, the samples were stored during 24 hours at room temperature, allowing the phthalates to penetrate into the material. The performance characteristics are listed in Table 15. The recoveries of the different phthalates in packaging materials ranged between 82% and 99% and were well reproducible (Official Journal of the European Union, 2002), as indicated by the RSD values, which were lower than 14%.

In addition, Table 15 illustrates the LOQs that were separately calculated for each phthalate compound and for each kind of (sub)group and/or matrix. LOQs varied between 5 and 145 µg/kg fat for high-fat food products, between 0.2 and 8.0 µg/kg fresh weight for low-fat foods, between 0.01 and 0.03 µg/kg fresh weight for aqueous-based beverages and finally, between 0.1 and 1.5 ng/cm² for packaging materials. Good linearity (R^2 values >0.9998) was obtained for concentrations between 0.05 µg/ml and 1.15 µg/ml for each phthalate compound.

Figure 5 shows GC-MS chromatograms of two aqueous-based beverage samples from the measurement campaign, namely of (A) a sparkling water sample packed in polyethylene terephthalate and (B) a canned beer sample. Both samples were spiked with 25 µl of the internal standard solution, as described in Section II.1.2.4. In the chromatogram of the water sample (Figure 5A), distinct peaks were observed for each phthalate compound, thereby enabling quantification. However, chromatograms of some food samples – especially of beer samples – were disturbed by matrix interferences, as illustrated in Figure 5B. This means that certain phthalate compounds could not univocally be identified and quantified in some of the investigated samples. Interferences in beer samples were typically present in the region of 18-21 min, which is the area where BBP (19.8 min), DEHP (20.6 min) and DCHP (20.9 min) elute from the column.

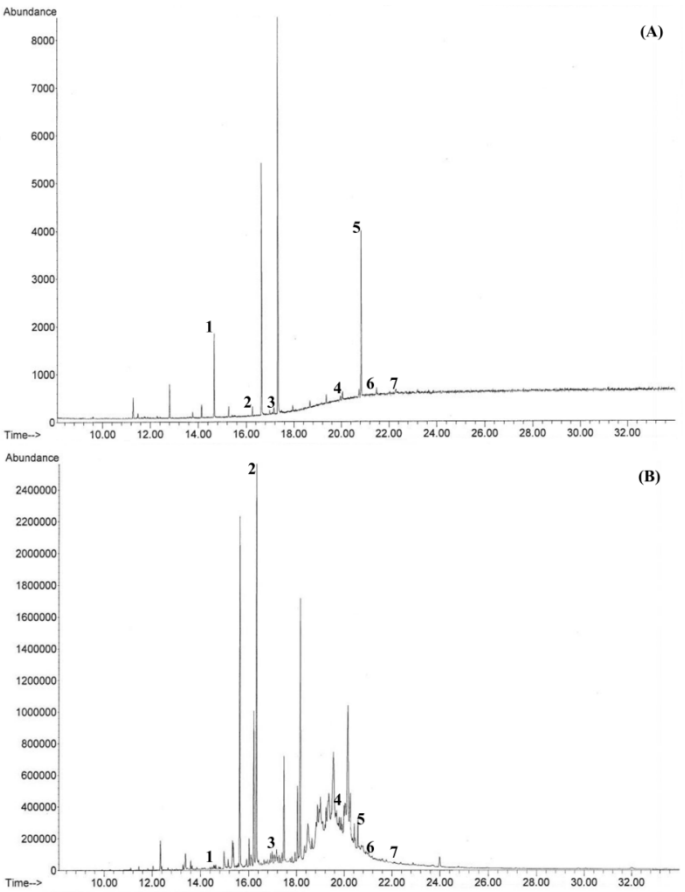

Figure 5: GC-MS chromatograms in SIM mode (m/z 149) of a water sample (A) and a beer sample (B), both spiked with 25 µl of the internal standard solution (x-axis: retention time (min) – y-axis: abundance (counts)). Numbering: (1) DEP (14.6 min), (2) DiBP (16.4 min), (3) DnBP (17.1 min), (4) BBP (19.8 min), (5) DEHP (20.6 min), (6) DCHP (20.9 min) and (7) DnOP (22.0 min).

II.1.3.2 Phthalate levels in food and packaging materials sold on the Belgian market

The number of positive samples for each phthalate compound in the twelve investigated groups is reported in Table 16. DEHP was the most detected phthalate compound (identified in 81% of the 400 samples), followed by DiBP (75%), DnBP (69%) and BBP (58%). On the contrary, DMP, DEP, DCHP and DnOP were seldom present in the investigated samples.

Phthalate concentrations observed in the different food groups are summarised in Table 17; concentrations per subgroup can be consulted in Table 53 (Annexes). In general, DEHP levels were the highest of all phthalate levels in every investigated group, notwithstanding the fact that for some particular food groups, high concentrations for other phthalate compounds were observed as well. For example, a condiment sample contained a DMP concentration of 4240 µg/kg fresh weight. High maximum concentrations of DEP and DiBP were detected in cereal and cereal product samples, more specifically in pasta and rice, respectively. A vegetable oil sample contained BBP in a concentration of 1130 µg/kg fresh weight. Of all food groups, the lowest phthalate concentrations were observed in baby food and aqueous-based beverages.

Table 16: Number of positive samples for each phthalate compound in the different food and packaging groups. The number of samples is given between brackets.

Group	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (27)	11	4	18	21	11	13	2	6
Milk and dairy products (56)	2	11	43	43	15	52	30	3
Cereals and cereal products (47)	13	26	44	35	38	45	6	20
Meat and meat products (22)	13	4	19	14	3	22	22	6
Fish and fish products (18)	9	10	8	8	4	17	1	4
Fat and oils (26)	4	6	8	4	22	18	1	0
Snacks (28)	3	9	25	24	17	26	4	4
Condiments and sauces (40)	13	7	23	29	34	39	5	7
Miscellaneous (22)	8	4	19	14	19	15	4	7
Baby food (17)	4	9	17	17	15	16	6	10
Beverages (85)	45	30	65	56	45	48	10	19
Packaging materials (12)	3	7	10	10	10	12	6	2
Total (400)	128	127	299	275	233	323	97	88
Total (%)	32%	32%	75%	69%	58%	81%	24%	22%

In packaging materials, especially in cardboard, phthalate contamination was primarily due to the presence of DiBP (Table 17; concentrations per subgroup can be found in Table 53 (Annexes)). This is not surprising, since this phthalate compound is often used as additive in printing inks and lacquers of food contact materials (BfR, 2007; CDC, 2009).

Table 17: Phthalate concentrations (min-max (median)) determined in every group. Concentrations in foods and beverages are reported in µg/kg fresh weight and concentrations in packaging materials in ng/cm². The number of samples is given between brackets in the first column.

Group	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (27)	ND-4.6 (ND)	ND-2.0 (ND)	ND-13 (1.0)	ND-17 (1.7)	ND-26 (ND)	ND-1413 (ND)	ND-0.5 (ND)	ND-0.9 (ND)
Milk and dairy products (56)	ND-0.5 (ND)	ND-11 (ND)	ND-116 (2.4)	ND-54 (2.0)	ND-8.2 (ND)	ND-743 (28)	ND-42 (0.4)	ND-5.7 (ND)
Cereals and cereal products (47)	ND-1.4 (ND)	ND-558 (0.5)	ND-1054 (8.7)	ND-61 (4.6)	ND-14 (1.5)	ND-1073 (63)	ND-3.6 (ND)	ND-2.8 (ND)
Meat and meat products (22)	ND-25 (2.0)	ND-1.4 (ND)	ND-9.7 (2.0)	ND-15 (1.5)	ND-18 (ND)	10-433 (45)	ND-2.0 (ND)	ND-51 (ND)
Fish and fish products (18)	ND-43 (ND)	ND-9.3 (0.6)	ND-13 (ND)	ND-13 (ND)	ND-8.0 (ND)	ND-5932 (86)	ND-0.1 (ND)	ND-0.8 (ND)
Fat and oils (26)	ND-32 (ND)	ND-154 (ND)	ND-53 (ND)	ND-203 (ND)	ND-1127 (13)	ND-1827 (102)	ND-13 (ND)	ND
Snacks (28)	ND-6.1 (ND)	ND-5.3 (ND)	ND-114 (4.3)	ND-65 (3.2)	ND-14 (0.6)	ND-308 (35)	ND-4.7 (ND)	ND-73 (ND)
Condiments and sauces (40)	ND-4238 (ND)	ND-84 (ND)	ND-155 (ND)	ND-157 (2.8)	ND-388 (2.3)	ND-2154 (44)	ND-2.8 (ND)	ND-120 (ND)
Miscellaneous (22)	ND-4.7 (ND)	ND-2.5 (ND)	ND-344 (3.3)	ND-28 (3.4)	ND-5.9 (0.8)	ND-718 (16)	ND-1.8 (ND)	ND-2.6 (ND)
Baby food (17)	ND-0.2 (ND)	ND-1.6 (0.1)	0.1-16 (2.7)	0.1-32 (1.3)	ND-16 (2.1)	ND-67 (22)	ND-1.8 (ND)	ND-3.0 (0.2)
Beverages (85)	ND-0.2 (0.1)	ND-0.3 (0.1)	ND-2.0 (0.1)	ND-2.1 (0.1)	ND-1.6 (0.1)	ND-11 (0.1)	ND-0.1 (ND)	ND-0.8 (ND)
Packaging materials (12)	ND-0.4 (ND)	ND-41 (3.8)	ND-523 (19)	ND-96 (22)	ND-24 (1.5)	1.1-319 (32)	ND-25 (0.1)	ND-1.5 (ND)

ND: not detected.

II.1.4 Discussion

II.1.4.1 Strengths and weaknesses

The research project PHTAL was the first national survey that investigated the occurrence of phthalate compounds in foodstuffs sold on the Belgian food market. In the measurement campaign of this project, phthalates were analysed in 400 different food and packaging samples present on the Belgian market. Therefore, suitable extraction techniques were developed and validated for four different matrices – high-fat foods, low-fat food products, aqueous-based beverages and packaging materials – in order to determine the concentrations of eight phthalate compounds via GC-EI-MS. Special attention was paid to the reduction of the risk of contamination during sample preparation and analysis.

Unfortunately, GC-MS chromatograms of some samples, especially beer samples, were disturbed by matrix interferences, which means that certain phthalate compounds – i.e. BBP, DEHP and DCHP – could not univocally be identified and quantified in these samples. Another analytical method and/or extraction technique might be a solution for the identification and quantification of these compounds in samples like beer.

II.1.4.2 Comparison with other studies

In this section, phthalate concentrations determined in this measurement campaign are compared with concentrations reported in literature. Thereby, only recent studies concerning phthalate levels in retail foods and packaging materials are considered. The comparison is made in chronological order. A summary of the phthalate concentrations reported in the different studies can be found in Table 18. Regrettably, due to the use of other units (e.g. µ*g/kg* in literature compared to *ng/dm²* in this study), phthalate levels in packaging materials reported in literature could not always be compared with the results obtained in the PHTAL project. In that case, phthalate concentrations are only mentioned.

Tsumura et al. (2001b) investigated the occurrence of phthalate compounds in a.o. 16 lunches, packed in polystyrene and purchased from Japanese shops. The determined concentrations of DEP, DnBP, DCHP and DnOP are similar to the concentrations of these phthalates in ready-to-eat meals determined in the PHTAL project. Conversely, Tsumura and colleagues observed larger BBP and DEHP concentrations ranges, namely 1.3–277 µg/kg fresh weight and 346–11,800 µg/kg fresh weight, respectively. The high levels of DEHP were mainly caused by DEHP containing PVC gloves used during the preparation of the foods. Two months after the prohibition of DEHP-containing PVC gloves for cooking purposes in Japan, Tsumura et al. (2001c) redetermined a.o. BBP and DEHP concentrations in 10 retail packed lunches. BBP and DEHP levels varied between not detected and 10 µg/kg fresh weight and between 45 and 517 µg/kg fresh weight, respectively, which is much lower than the previous determined levels and which is more comparable to the concentrations of BBP and DEHP determined in the PHTAL project. In another survey of Tsumura et al. (2002b), a.o. DnBP, BBP and DEHP were determined in 93 Japanese retail foods. Median levels obtained in this Japanese survey are, in general, comparable to results from the PHTAL project.

Table 18: Overview of phthalate concentrations (min-max (median)) in food products and packaging materials obtained in several studies.

Study	Country	N	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
						In µg/kg fresh weight				
Fruits and vegetables										
Pfördt, 2004	Germany	5	-	-	ND-100 (ND)	ND-170 (ND)	-	60-1580 (230)	-	-
This study	Belgium	27	ND-4.6 (ND)	ND-2.0 (ND)	ND-13 (1.0)	ND-17 (1.7)	ND-26 (ND)	ND-1413 (ND)	ND-0.5 (ND)	ND-0.9 (ND)
Milk and dairy products										
Tsumura et al., 2002a	Japan	9	-	-	-	ND	ND-8	63-570	-	-
Pfördt, 2004	Germany	10	-	-	ND-730 (ND)	ND-70 (ND)	-	120-920 (330)	-	-
Peters, 2006	Europe	8	ND	ND-5.6 (ND)	ND-4400 (ND)	ND-780 (104)	ND-50 (23)	ND-3000 (490)	-	-
This study	Belgium	56	ND-0.5 (ND)	ND-11 (ND)	ND-116 (2.4)	ND-54 (2.0)	ND-8.2 (ND)	ND-743 (28)	ND-42 (0.4)	ND-5.7 (ND)
Cereals and cereal products										
Pfördt, 2004	Germany	6	-	-	ND-10 (ND)	ND-20 (ND)	-	40-750 (115)	-	-
This study	Belgium	47	ND-1.4 (ND)	ND-558 (0.5)	ND-1054 (8.7)	ND-61 (4.6)	ND-14 (1.5)	ND-1073 (63)	ND-3.6 (ND)	ND-2.8 (ND)
Meat and meat products										
Pfördt, 2004	Germany	10	-	-	ND	ND-130 (ND)	-	ND-280 (ND)	-	-
Peters, 2006	Europe	10	ND-4.9 (ND)	ND-24 (ND)	ND-2300 (ND)	ND-760 (ND)	ND-17 (ND)	ND-3300 (250)	-	-
This study	Belgium	22	ND-25 (2.0)	ND-1.4 (ND)	ND-9.7 (2.0)	ND-15 (1.5)	ND-18 (ND)	10-433 (45)	ND-2.0 (ND)	ND-51 (ND)
Fat and oils										
Tsumura et al., 2002a	Japan	17	-	-	-	ND-2400	ND-620	ND-2830	-	-
Peters, 2006	Europe	1	ND	ND	ND	ND	340	24000	-	-
This study	Belgium	26	ND-32 (ND)	ND-154 (ND)	ND-53 (ND)	ND-203 (ND)	ND-1127 (13)	ND-1827 (102)	ND-13 (ND)	ND
Snacks										
Tsumura et al., 2002a	Japan	9	-	-	-	ND-70	ND	ND-680		
This study	Belgium	28	ND-6.1 (ND)	ND-5.3 (ND)	ND-114 (4.3)	ND-65 (3.2)	ND-14 (0.6)	ND-308 (35)	ND-4.7 (ND)	ND-73 (ND)
Ready-to-eat meals										
Tsumura et al., 2001a	Japan	16	-	ND-1.6 (ND)	-	ND-90 (12)	1.3-277 (6.4)	346-11800 (3300)	-	ND-3.6 (ND)
Tsumura et al., 2001b	Japan	10	-	ND	-	ND	ND-10 (1.7)	45-517 (128)	-	ND
This study	Belgium	22	ND-4.7 (ND)	ND-2.5 (ND)	ND-344 (3.3)	ND-28 (3.4)	ND-5.9 (0.8)	ND-718 (16)	ND-1.8 (ND)	ND-2.6 (ND)

Study	Country	N	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Baby food										
Tsumura et al., 2002a	Japan	24	-	-	-	ND-250	ND-3	ND-1830	-	-
This study	Belgium	17	ND-0.2 (ND)	ND-1.6 (0.1)	0.1-16 (2.7)	0.1-32 (1.3)	ND-16 (2.1)	ND-67 (22)	ND-1.8 (ND)	ND-3.0 (0.2)
Beverages										
Tsumura et al., 2002a	Japan	11	-	-	-	ND-660	ND-2	ND-27	-	-
Peters, 2006	Europe	1	ND	ND	ND	ND	ND	ND	-	-
Bosnir et al., 2007	Croatia	45	ND-3000	ND-200	-	ND-133	ND-27	ND-136	-	ND
This study	Belgium	85	ND-0.2 (0.1)	ND-0.3 (0.1)	ND-2.0 (0.1)	ND-2.1 (0.1)	ND-1.6 (0.1)	ND-11 (0.1)	ND-0.1 (ND)	ND-0.8 (ND)
Packaging materials										
						In µg/kg				
Jarosova, 2006	Czech Republic	42	-	-	-	100-1.3E6	-	100-4.3E6	-	-
Lopez-Espinosa et al., 2007	Spain	40	-	-	-	0.1-1.1E4 (122)	-	0.5-6.1E4 (894)	-	-
Zhang et al., 2008	USA	69	-	-	-	140-5.5E4	-	-	-	-
Poças et al., 2010	Portugal	21	-	ND-280 (ND)	ND-2.1E4 (4000)	ND-2300 (790)	-	460-5100 (2800)	-	-
						In ng/cm²				
Bononi and Tateo, 2009	Italy	16	-	-	2.5-28 (5.9)	-	-	-	-	-
This study	Belgium	12	ND-0.4 (ND)	ND-41 (3.8)	ND-523 (19)	ND-96 (22)	ND-24 (1.5)	1.1-319 (32)	ND-25 (0.1)	ND-1.5 (ND)

N: number of samples; ND: not detected; -: not investigated.

Food samples packed in plastic and purchased from German retail shops, were examined for the occurrence of DEHP, DnBP and DiBP (Pfördt, 2004). The 31 investigated food products included ham sausage, minced meat, cheese, rye bread and hazelnuts. Concentrations of DEHP, DnBP and DiBP varied between not detected and 1,580; 170 and 730 µg/kg fresh weight, respectively, which is in line with the concentrations that were found in the PHTAL project.

In a Swiss study, 158 fatty foods packed in glass jars were analysed for the presence of a.o. DEHP (Fankhauser-Noti et al., 2006). Maximum concentrations – only the seven highest detected DEHP concentrations were mentioned in the publication – varied between 360 and 825 mg/kg fresh weight, which is about 100 times higher than the maximum concentration that was observed for DEHP in this study. This contrast probably arises from the use of other sampling strategies in the two studies. To be precise, only food samples of which it was expected that they contained a lot of DEHP were investigated in the Swiss study, while in the PHTAL project, food samples that are representative for the diet of the whole Belgian population were also considered.

Jarosova (2006) investigated the occurrence of both DnBP and DEHP in food products and packaging materials available on the Czech market. Overall, Czech food contains more DnBP (<10–1,310 µg/kg fresh weight), but less DEHP (<10–220 µg/kg fresh weight) than Belgian food products. DnBP concentrations in printed packaging materials varied between 0.1 and 1,298 mg/kg packaging, while DEHP levels ranged from 0.1 to 4,259 mg/kg packaging.

DnBP, BBP and DEHP were analysed by Peters (2006) in 27 daily consumed European foodstuffs. Levels of DnBP varied between 76 and 780 µg/kg fresh weight, which is higher than the concentrations observed for DnBP in this study. Apart from the exceptional high DEHP concentration of 24,000 µg/kg fresh weight in one sample of olive oil, BBP and DEHP levels are comparable to concentrations determined in this study, namely 2–340 µg/kg fresh weight and 20–3,300 µg/kg fresh weight, respectively.

The presence of DMP, DEP, DnBP, BBP, DEHP and DnOP was examined in 45 soft drinks and mineral waters available on the Croatian market (Bosnir et al., 2007). Of all investigated phthalate compounds, the highest concentration was found for DMP in a sample of soft drink, namely 3,000 µg/l, which is a lot more than the maximum DMP concentration of 0.2 µg/kg fresh weight that was found for beverages in the PHTAL study. Levels of DEP, DnBP, BBP and DEHP were ranging from not detected to 200, 133, 27 and 136 µg/l, respectively. Also for these four compounds, the conclusion can be made that Croatian beverages contain more phthalates than beverages available on the Belgian market. DnOP concentrations were, just like in the PHTAL project, not detected.

In a Spanish study, DnBP and DEHP levels were determined in 40 paper and cardboard containers used for take-away food originating from four different European countries (Lopez-Espinosa et al., 2007). Concentrations of DnBP ranged from 0.10 to 10,744 µg/kg packaging with a median level of 122 µg/kg packaging. DEHP levels varied between 0.52 and 61,013 µg/kg packaging (median level of 894 µg/kg packaging).

In the United States of America, food and paper packaging materials were examined for the occurrence of a.o. DnBP (Zhang et al., 2008). In food, DnBP concentrations varied between <10 and 810 µg/kg fresh weight. A smaller range was obtained in the PHTAL project (not detected – 203 µg/kg

fresh weight), which could be ascribed to the fact that food available on the European market have to comply with another legislation than food available in the United States of America (Schäfer, 2007; Twaroski et al., 2007). Concentrations of DnBP in American packaging materials varied between 140 and 55,000 µg/kg packaging.

Bononi and Tateo (2009) identified the release of DiBP in 16 Italian recycled take-away pizza boxes. The migration of DiBP from the boxes varied between 2.5 and 28 ng/cm² while in the PHTAL project, concentrations of DiBP between not detected and 523 ng/cm² were found. However, it should be noticed that the analytical procedure that was conducted in the Italian study – i.e. migration tests – is different from the method carried out in the PHTAL project.

Finally, in a Portuguese survey, Poças and co-workers (2010) explored the occurrence of phthalate compounds – including DEP, DiBP, DnBP and DEHP – in 21 cellulosic materials as well as in the food products packed in these materials. The investigated foodstuffs were mostly dried foods (e.g. pasta, cereals and cookies). DEP and DnBP were not detected, while concentrations of DiBP and DEHP varied between not detected and 360 and 2,200 µg/kg fresh weight, respectively. Phthalate concentrations in Portuguese foods are thus similar to concentrations in food products available on the Belgian market. Regarding packaging materials, DEHP was detected in all the investigated packages in a concentration range of 460–5,100 µg/kg packaging. Concentrations of DEP, DiBP and DnBP varied between not detected and 280; 21,000 and 2,300 µg/kg packaging, respectively.

II.1.4.3 Future research

In a next phase of the PHTAL project, the results obtained in this measurement campaign will be evaluated in detail. Moreover, a second, more targeted, measurement campaign will be set up (see Chapter II.2). This extra campaign will focus on remarkable results of the first measurement campaign and possible contamination routes for phthalates in food products available on the Belgian market. Finally, the results of both measurement campaigns will be combined with data on food consumption from Belgian food consumption surveys (Devriese et al., 2006; Huybrechts et al., 2008b) to estimate the dietary exposure to phthalates in the Belgian population (see Chapter II.3).

II.1.5 Conclusions

In this survey, the occurrence of eight phthalate compounds – DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP – was measured in 400 food products, divided over eleven groups, and packaging materials sold on the Belgian market. Therefore, suitable extraction techniques were developed and validated for four different matrices (high-fat foods, low-fat foods, aqueous-based beverages and packaging materials). In this measurement campaign, DEHP was the most detected phthalate compound, followed by DiBP, DnBP and BBP. Phthalate concentrations differed from group to group, although, in general, DEHP levels were the highest of all phthalate levels in every investigated group. The results obtained in this study are part of the PHTAL project, the first project that discusses phthalate contamination on the Belgian food market.

Supplementary data

Supplementary data can be found in Table 53 (Annexes).

II.2 PHTAL 2 – Contamination pathways of phthalates in the Belgian food chain

Van Holderbeke, M., Geerts, L., Vanermen, G., Servaes, K., Sioen, I., De Henauw, S. and Fierens, T. (2014). ***Determination of contamination pathways of phthalates in food products sold on the Belgian market.*** *Environmental Research, 134, 345–352.*

Abstract

As numerous studies have indicated that food ingestion is the most important exposure pathway to several phthalates, this study aimed to determine possible contamination pathways of phthalates in food products sold on the Belgian market. To do this, concentrations of eight phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), dicyclohexyl phthalate (DCHP), di(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP)) were determined in 591 foods and 30 packaging materials. In general, the four most prominent phthalates in Belgian food products were DEHP, DiBP, DnBP and BBP. Special attention was given to the origin of these phthalates in bread, since high phthalate concentrations (especially DEHP) were determined in this frequently consumed food product (see Chapter II.1). Phthalates seemed to occur in Belgian bread samples due to the use of contaminated ingredients (i.e. use of contaminated flour) as well as due to migration from phthalate containing contact materials used during production (e.g. coated baking trays). Also the results of the conducted concentration profiles of apple, bread, salami and two cheese types revealed the important role of processing – and not packaging – on phthalate contents in foods.

II.2.1 Introduction

Phthalates (diesters of ortho-phthalic acid) are a group of organic lipophilic chemicals with a wide range of user applications. They are primarily used as plasticisers to increase the elasticity of polymer products and can be present in printing inks, lacquers, building materials, pharmaceutical products (e.g. enteric-coated tablets) and medical devices (e.g. blood bags and tubings). Due to their widespread use, phthalates are omnipresent in the environment (Cao, 2010; Fromme et al., 2007a; Wittassek et al., 2011; Wormuth et al., 2006).

Over the last decades, phthalates have attracted public attention due to their possible harmful effects to human health (Hauser and Calafat, 2005; Meeker et al., 2009; Shea, 2003). For example, some phthalates and their metabolites have been reported to adversely affect the male reproductive system (Meeker et al., 2009) and to have the potential to alter androgen-responsive brain development in humans (Swan et al., 2010). Of all exposure pathways, food intake is the most important one for many phthalates, followed by dust ingestion and indoor air inhalation (Clark et al., 2011; Fromme et al., 2007b; Rudel and Perovich, 2009; Wormuth et al., 2006).

Between 2009 and 2011, a Belgian research project (acronym: PHTAL) was conducted by order of the Belgian Federal Public Service of Health, Food Chain Safety and Environment with the following main objectives:

- To obtain accurate and sensitive data of phthalates in all kinds of food products and packaging materials sold on the Belgian market;
- To gain a clear understanding of possible contamination pathways of phthalates in the Belgian food market;
- To estimate dietary exposure to phthalates in the Belgian population.

In this PHTAL project, eight frequently used phthalate compounds were considered: dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), dicyclohexyl phthalate (DCHP), di(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP).

The first and third objectives of the PHTAL project have been published recently by Fierens et al. (2012c) and Sioen et al. (2012), respectively. To investigate the occurrence of phthalates in food products and packaging materials on the Belgian market (i.e. the first objective; see also Chapter II.1), an analytical procedure was developed and validated for the determination of the eight phthalate compounds of interest in various types of foods and packaging materials. Subsequently, a first screening measurement campaign (MC1) was conducted between 2009 and 2010, in which the phthalate compounds were analysed in 388 food products and 12 packaging materials. Sample selection for MC1 was based on consumption data from the most recent Belgian national food consumption survey (De Vriese et al., 2005) and the likelihood that foodstuffs contain phthalates. During MC1, DEHP was observed to be the most abundant phthalate compound, followed by DiBP, DnBP and BBP. In food products, concentrations of DEHP were generally also the highest of all considered phthalate compounds. DEHP levels above 1,000 µg/kg fresh weight were among other things determined in bread, vegetable oil, (goat's) cheese and fish products. In packaging materials on the other hand, DiBP concentrations were the highest, especially in cardboard (Fierens et al., 2012c).

To have an idea of the dietary phthalate exposure in the Belgian population (i.e. the third objective; see also Chapter II.3), the analysed phthalate concentrations in foods were linked to data from two Belgian food consumption surveys: one for preschool children (Huybrechts et al., 2008a) and one for adults (De Vriese et al., 2005). For both preschool children and adults, dietary intake of DEHP was the highest, followed by DiBP. The estimated intake values of DEP, DnBP and BBP were far below the tolerable daily intake (TDI) values (EFSA, 2005a; 2005b; WHO, 2003). However, for DEHP, the 99th percentile of the intake distribution of preschoolers in the worst case scenario was equal to 80% of the TDI (EFSA, 2005c). Bread was observed to be the most important contributor to dietary DEHP exposure in the Belgian population (Sioen et al., 2012).

This paper describes the results of the second research objective of the PHTAL project, namely the determination of contamination pathways of phthalates in food products sold on the Belgian market. Thereto, a second, more oriented measurement campaign (MC2) was conducted, in which 203 extra food products and 18 extra packaging materials were analysed. The sample selection of MC2 was mainly based on the measurement results of MC1: food products, in which high phthalate contents

were determined (e.g. bread) and were now investigated in more detail. Food products not considered in MC1, but necessary to assess dietary intake (e.g. eggs, coffee and vegetarian food) were analysed in MC2 as well.

II.2.2 Material and methods

II.2.2.1 Sample collection

MC2 of the PHTAL project was carried out between 2010 and 2011. During this campaign, 203 additional food products and 18 additional packaging materials were purchased from various Belgian shops. Samples were collected in order to (1) gain more insight into possible contamination pathways of phthalates in the Belgian food chain, especially of the food items that contained high phthalate concentrations in MC1 of the project (e.g. bread) (Fierens et al., 2012c) and (2) to complete the concentration database to correctly assess the dietary intake of phthalates in the Belgian population (Sioen et al., 2012). Information regarding brand name, packaging material and properties, fat content, shelf life, time and place of purchase, picture and – if relevant – product specific properties (e.g. pH and preserving agents) of food were stored in a database. An overview of the sample selection of MC2 is given in Table 19. For comparison, the numbers of samples collected during MC1 of the PHTAL project (Fierens et al., 2012c) are also mentioned.

Besides giving an overview of the general analytical results of the food groups additionally considered in MC2 (i.e. wine, coffee and tea, vegetarian food (i.e. meat substitutes), eggs and boiling water (i.e. tap water in which pasta or rice was boiled)), two topics were investigated in this study in more detail for the four most detected phthalate compounds, namely (1) concentrations in bread: different types of bread, different providers, different packaging materials, concentrations in bread ingredients versus bread, and so on, and (2) concentration profiles in different food items (e.g. salami, goat's cheese and apple).

Table 19: Overview of the selected food products and packaging materials in the first (MC1) and second measurement campaign (MC2) of the PHTAL project. The number of samples is given for each campaign separately as well as for the total campaign.

Group – Subgroup	Total	MC1[a]	MC2
Fruits and vegetables	*47*	*27*	*20*
Fruits	17	7	10
Vegetables	24	17	7
Nuts	6	3	3
Milk and dairy products	*79*	*56*	*23*
Milk	9	8	1
Milk beverages	10	8	2
Cheese	38	21	17
Fresh cheese, yoghurt, cream, dessert, milk pudding, etc.	22	19	3
Cereals and cereal products	*129*	*47*	*82*
Bread	62	18	44
Breakfast cereals	7	7	0
Pasta	21	11	10
Rice	19	4	15
Flour, starches, pudding powder, couscous, popcorn and oatmeal	20	7	13

Group – Subgroup	Total	MC1[a]	MC2
Meat and meat products	*37*	*22*	*15*
Meat	17	13	4
Meat products	20	9	11
Fish and fish products	*22*	*18*	*4*
Fish	14	10	4
Fish products	6	6	0
Crustaceans	2	2	0
Fat and oils	*34*	*26*	*8*
Vegetable oils	21	15	5
Vegetable fat	9	8	2
Animal fat	4	3	1
Snacks	*29*	*27*	*2*
Salty biscuits	4	4	0
Sweet biscuits and cakes	10	10	0
Confectionery	5	4	1
Syrup, sugar, honey, popcorn, chocolate spread, cocoa, etc.	10	9	1
Condiments and sauces	*41*	*40*	*1*
Condiments	7	7	0
Pesto	4	4	0
Mayonnaise	7	6	1
Mustard, vinaigrette, ketchup, curry, etc.	23	23	0
Miscellaneous	*56*	*22*	*34*
Ready-to-eat meals raw/cold	39	22	17
Ready-to-eat meals prepared	17	0	17
Baby food	*17*	*17*	*0*
Milk powder	3	3	0
Fruit puree, vegetable puree, soup, etc.	14	14	0
Beverages	*89*	*85*	*4*
Beer	18	18	0
Wine	2	0	2
Soft drinks	25	25	0
Juices	22	22	0
Water	20	20	0
Coffee and tea	2	0	2
Vegetarian food	*5*	*1*	*4*
Meat substitutes	3	0	3
Milk substitute	2	1	1
Eggs	*2*	*0*	*2*
Boiling water (pasta/rice)	*4*	*0*	*4*
Packaging materials	*30*	*12*	*18*
Cardboard	7	5	2
Tetra brick	2	2	0
Plastic	9	5	4
Multi-layer	5	0	5
Wax	1	0	1
Paper	6	0	6

[a] Fierens et al. (2012c).

a) Bread

In MC1, it was demonstrated that bread samples had a relatively high DEHP content, i.e. DEHP levels up to 1,073 µg/kg were noticed (Fierens et al., 2012c). Because the large share of bread in the food consumption pattern of children and adults (Devriese et al., 2006; Vanhauwaert, 2012), additional bread products were bought in MC2 of the PHTAL project in order to investigate possible contamination sources. Of all bread products analysed in both MC1 and MC2 (n=62), 43 bread samples were investigated in more detail. These bread samples were purchased at different locations spread over Belgium and were originating from supermarket chains as well as from fresh bakeries. Parameters that were explored in these 43 bread samples were: flour type (white (n=21) versus brown/wholemeal (n=22)), packaging type (paper (n=32) versus paper with plastic window (n=5) versus plastic (n=6)), location (nine fresh bakery locations (n=20) versus twelve supermarket locations (n=23)), form (long (n=31) versus round (n=12)) and contact time with bread bag (long/prepacked (n=7) versus short/packed at purchase (n=36)).

By comparing absolute phthalate contents of a bread bag with absolute phthalate contents of the bread samples that were packed in this bread bag, the role of the packaging material on phthalate contamination in bread could be investigated. For this purpose, phthalate concentrations were determined in two supermarket bread samples with high DEHP contents (i.e. 1,002 and 1,073 µg/kg fresh weight) as well as in the paper bag of these two bread samples. Subsequently, absolute phthalate contents in the bread bag and in the two bread samples were calculated by multiplying the analysed concentrations by an assumed contact surface of 1,200 cm^2 and a bread weight of 0.8 and 0.6 kg fresh weight, respectively.

To investigate whether phthalates may transfer from contact materials to bread during baking and/or whether phthalate contamination may arise from the ingredients used, three homemade bread samples were analysed as well as the flour mixes used to bake these breads. The first brown bread was baked in a bread machine with a coated metal baking tray using 500 g of an all-in bread mix (i.e. a mix of flour, flour treatment agent, ascorbic acid and enzymes) and tap water. The second wholemeal bread was baked in a blue-steel baking tray in a conventional hot-air oven after mixing 500 g of a wholemeal flour mix (flour, flour treatment agent, and ascorbic acid), 30 g of margarine, dried yeast, salt and tap water. Mixing of these ingredients took place in a metal pot with a metal kneader. The third bread was baked in a metal baking tray coated with polytetrafluoroethylene in a conventional hot-air oven. This dark bread was composed of 500 g of a Black Forest flour mix (mixture of three kinds of flour), 30 g of margarine, yeast, salt and tap water. All three breads were unsliced prior to analysis. Just like in the previous mentioned investigation, absolute phthalate contents were calculated in order to compare phthalate contents in the bread and corresponding flour mix samples. To do this, the phthalate concentrations in the bread samples were multiplied by a weight of 0.8 kg and the phthalate levels in the flour mix samples were multiplied by a weight of 0.5 kg.

b) Concentration profiles

"Concentration profiles" of five different food products (apple, bread, soft goat's cheese, salami and semi-soft cheese) were established in order to investigate the origin of the phthalates detected (i.e. migration from contact materials, environmental transfer and/or the production process). Thereto, samples were taken from the surface and in the core of apple and bread. Regarding soft goat's cheese, salami and semi-soft cheese, samples were taken from different places in the product as shown in Figure 6. All samples were collected using a kitchen knife that was rinsed with dichloromethane (Merck, Overijse, Belgium) prior to sampling. Regarding the packaging materials of the investigated foods, apple was not packed and bread and salami were packed at purchase in a paper bag and in paper with a polyethylene lining, respectively. Soft goat's cheese was packed in a plastic printed tub with on the inside of the bottom, an extra metallic layer and the investigated semi-soft cheese was wrapped in wax with on the outside, an additional printed plastic layer.

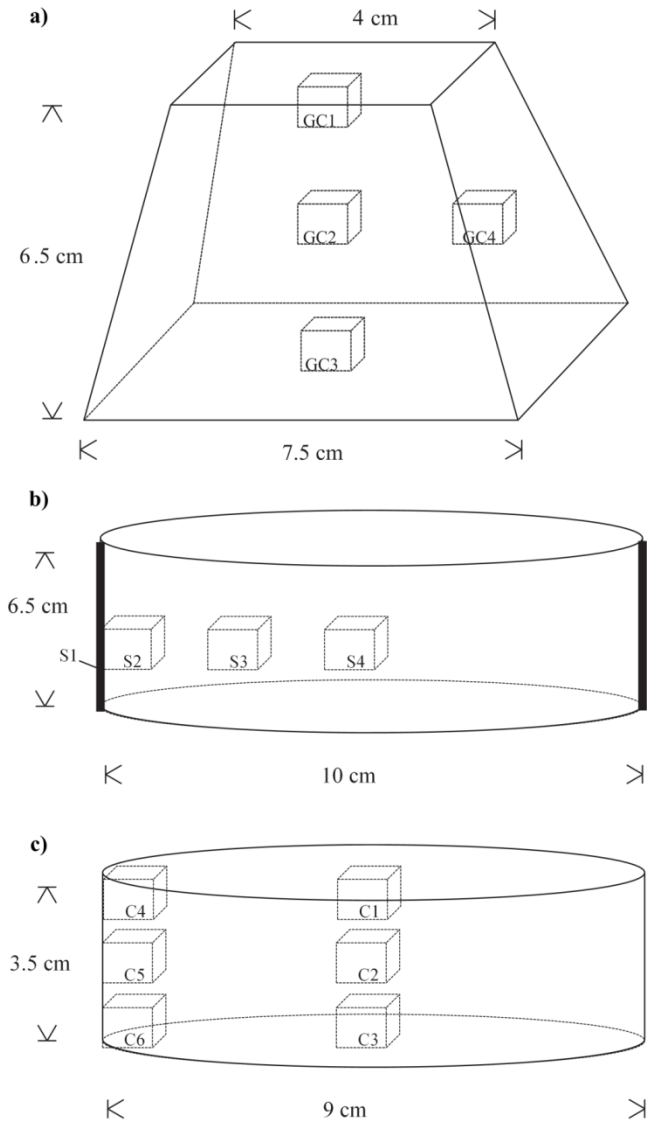

Figure 6: Schematic overview of the considered sampling places for the concentration profiles of a) soft goat's cheese (GC), b) salami (S) and c) semi-soft cheese (C).

II.2.2.2 Analytical procedure

The concentrations of DMP, DEP, DnBP, DiBP, BBP, DEHP, DCHP and DnOP in all food products and packaging materials of MC2 were analysed in the same way as the samples analysed in MC1 of this project (Fierens et al., 2012c). In brief, for the sample preparation, a distinction was made between high-fat foods (fat content of more than one percent on a fresh weight basis), low-fat foods (fat content of less than one percent), aqueous-based beverages and packaging materials. Of every high-fat food sample, 5 to 20 g was extracted with acetone/*n*-hexane (1:1; Merck, Overijse, Belgium) to obtain at least 0.5 g fat; for the extraction of low-fat foods, 10 g was used. For aqueous-based beverages, a liquid–liquid extraction with dichloromethane was applied using 500 ml of each sample. Packaging materials were cut into pieces of about 1 cm^2 and a representative subsample of 5 cm^2 was extracted with *n*-hexane. Subsequently, the sample extracts were exchanged to dichloromethane and were purified via gel permeation chromatography. The instrumental analysis of phthalates was performed by gas chromatography– low resolution-mass spectrometry with electron impact ionisation. Detection of the different phthalate compounds took place in single ion monitoring mode. For all phthalates except DMP, the dominant product ion *m/z* 149 was used as target ion; for DMP, *m/z* 163 was used. As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compound in a sample had to be within 20% of that observed in a standard solution. Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (twelve samples at most) to reduce the risk of contamination. The analytical procedure was proven to be well reproducible. Recoveries varied between 88 and 104% and the relative standard deviation for each phthalate compound in the different matrices was lower than 13%. Measurement uncertainty was lower than 30% for each compound.

II.2.2.3 Reporting of analytical results

Phthalate concentrations were expressed in micrograms per kilogram fresh weight for foodstuffs and beverages (µg/kg fresh weight) and as nanograms per square centimetre for packaging materials (ng/cm^2).

Limits of quantification (LOQs) were strongly dependent on the feasible blank concentrations, since phthalates are omnipresent in the laboratory (Fierens et al., 2012c). Therefore, LOQ values were calculated based on the phthalate concentrations detected in the procedural blanks. The LOQ of each individual phthalate compound equalled the sum of the average blank concentration and six times the standard deviation of replicate procedural blank measurements under reproducibility conditions (each replicate determination was obtained from an independent extraction) and varied – for MC1 and MC2 together – between 5 and 130 µg/kg fat for high-fat foods, between 0.5 and 9 µg/kg fresh weight for low-fat foods, between 0.01 and 0.03µg/kg fresh weight for aqueous-based beverages and between 0.1 and 1.5 ng/cm² for packaging materials.

II.2.3 Results

II.2.3.1 General analytical results

Table 20 summarises the number of positive samples that were determined for each phthalate compound in the different food and packaging groups. As can be noticed, DEHP was the most detected phthalate compound in milk and dairy products (72/79), meat and meat products (35/37), fish and fish products (18/22), snacks (27/29), condiments and sauces (40/41) and in packaging materials (30/30). In vegetarian food, both DEHP and DiBP were the most prominent compounds as they were detected in all five investigated samples. DiBP was also detected the most in the following food groups: fruits and vegetables (31/47), cereals and cereal products (124/129), miscellaneous foods (50/56), baby food (together with DnBP, i.e. detected in all 17 samples), beverages (68/89) and boiling water of pasta and rice (together with DEP and DnBP, i.e. 100% detectable). In fat and oils on the other hand, the number of positive samples was the highest for BBP, namely 26 out of 34 samples.

Table 20: Number of positive samples for each phthalate compound in the different food and packaging groups for the two measurement campaigns together. The number of samples is given within parentheses.

Group	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (47)	20	17	31	30	26	28	6	14
Milk and dairy products (79)	7	20	65	58	26	72	32	9
Cereals and cereal products (129)	27	91	124	99	99	110	13	48
Meat and meat products (37)	24	10	32	23	5	35	2	12
Fish and fish products (22)	11	11	9	9	5	18	1	6
Fat and oils (34)	4	7	11	7	26	25	4	2
Snacks (29)	4	9	26	25	19	27	6	6
Condiments and sauces (41)	13	7	23	29	34	40	5	8
Miscellaneous (56)	35	17	50	47	40	42	5	21
Baby food (17)	4	9	17	17	15	16	6	10
Beverages (89)	49	32	68	58	49	51	13	21
Vegetarian food (5)	2	4	5	4	2	5	1	1
Eggs (2)	0	0	0	0	0	0	0	0
Boiling water (pasta/rice) (4)	3	4	4	4	3	3	1	1
Packaging materials (30)	4	11	28	27	21	30	10	5
Total (621)	207	249	493	437	370	502	105	164
Total (%)	33	40	79	70	60	81	17	26

Phthalate concentrations observed in the two measurement campaigns are summarised in Table 21; concentrations per subgroup can be consulted in Table 54 (Annexes). New food groups that were considered in MC2 towards MC1 were wine, coffee and tea, vegetarian food (i.e. meat substitutes), eggs and boiling water of pasta and rice (Table 19). Median concentrations of phthalates in coffee and tea were in line with levels observed in other beverage subgroups Table 54 (Annexes). On the contrary, levels of DnBP and BBP in wine were higher than in other beverages Table 54 (Annexes). With regard to the investigated boiling water samples of pasta and rice, higher median concentrations of DEP, DiBP, DnBP and DEHP were observed compared to ordinary water samples Table 54 (Annexes). In vegetarian food, phthalate concentrations were low with the highest concentrations determined for DEHP. In eggs, none of the considered phthalate compounds were detected (Table 21).

Table 21: Phthalate concentrations (min-max (median)) determined in every group for the two measurement campaigns together. Concentrations in foods and beverages are reported in µg/kg fresh weight and concentrations in packaging materials in ng/cm². The number of samples for every group is given within parentheses.

Group	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (47)	ND-13 (ND)	ND-889 (ND)	ND-480 (1.1)	ND-38 (1.4)	ND-58 (0.3)	ND-1413 (16)	ND-1.4 (ND)	ND-9.5 (ND)
Milk and dairy products (79)	ND-1.7 (ND)	ND-11 (ND)	ND-116 (3.6)	ND-54 (2.1)	ND-48 (ND)	ND-2385 (100)	ND-42.0 (ND)	ND-5.7 (ND)
Cereals and cereal products (129)	ND-3.8 (ND)	ND-558 (1.4)	ND-1383 (6.0)	ND-106 (4.2)	ND-24 (0.9)	ND-2264 (49)	ND-3.6 (ND)	ND-6.1 (ND)
Meat and meat products (37)	ND-26 (0.6)	ND-11 (ND)	ND-36 (3.0)	ND-25 (1.5)	ND-18 (ND)	10-850 (41)	ND-2.0 (ND)	ND-51 (ND)
Fish and fish products (22)	ND-43 (0.1)	ND-9.3 (0.2)	ND-13 (ND)	ND-13 (ND)	ND-8.0 (ND)	ND-5932 (36)	ND-0.1 (ND)	ND-0.8 (ND)
Fat and oils (34)	ND-32 (ND)	ND-154 (ND)	ND-53 (ND)	ND-203 (ND)	ND-1127 (11.5)	ND-1827 (93)	ND-13 (ND)	ND
Snacks (29)	ND-6.1 (ND)	ND-5.3 (ND)	ND-114 (4.5)	ND-65 (4.4)	ND-23 (0.7)	0.5-308 (55)	ND-4.7 (ND)	ND-73.0 (ND)
Condiments and sauces (41)	ND-4238 (ND)	ND-84 (ND)	ND-155 (ND)	ND-157 (2.7)	ND-388 (2.2)	ND-2154 (44)	ND-2.8 (ND)	ND-120 (ND)
Miscellaneous (56)	ND-7.6 (0.2)	ND-143 (ND)	ND-344 (2.7)	ND-55 (3.2)	ND-5.9 (0.7)	ND-718 (21)	ND-1.8 (ND)	ND-15 (ND)
Baby food (17)	ND-0.2 (ND)	ND-1.6 (0.1)	0.1-16 (2.7)	0.1-32 (1.3)	ND-16 (2.1)	ND-67 (22)	ND-1.8 (ND)	ND-3.0 (0.2)
Beverages (89)	ND-0.2 (0.1)	ND-0.3 (ND)	ND-2.0 (0.1)	ND-30 (0.1)	ND-21 (0.1)	ND-11 (0.1)	ND-0.1 (ND)	ND-0.8 (ND)
Vegetarian Food (5)	ND-4.6 (ND)	ND-2.4 (1.1)	3.0-7.5 (4.3)	ND-6.4 (2.6)	ND-12 (ND)	1.0-76 (13)	ND-2.7 (ND)	ND-3.3 (ND)
Eggs (2)	ND	ND	ND	ND	ND	ND	ND	ND
Boiling water pasta / rice (4)	ND-0.1 (0.1)	0.2-32 (1.8)	0.1-16 (2.6)	0.1-3.2 (0.9)	ND-1.1 (0.1)	ND-12 (1.1)	ND-0.1 (ND)	ND-0.1 (ND)
Packaging materials (30)	ND-0.4 (ND)	ND-49 (ND)	ND-523 (7.5)	ND-96 (6.3)	ND-28 (0.71)	1.1-482 (35)	ND-25 (ND)	ND-2.0 (ND)

ND: not detected.

II.2.3.2 Bread

In MC1, it turned out that bread contained high phthalate concentrations, in particular DEHP (Fierens et al., 2012c). Therefore, a more detailed analysis of the occurrence of the four most prominent phthalates (DiBP, DnBP, BBP and DEHP) in 43 purchased bread samples was conducted, taking into account different parameters: flour type, packaging type, location, form and contact time with bread bag. The analytical results of this investigation are summarised in Table 22. The highest concentrations of DiBP and DnBP were observed in a round, brown/wholemeal bread sample packed at purchase in paper and purchased at a fresh bakery and a supermarket, respectively. Regarding BBP and DEHP, the highest concentrations were measured in a long, white and brown/wholemeal bread sample, respectively; both bought from a fresh bakery and packed at purchase in a paper bag. Of the investigated parameters, only the location considerably influenced phthalate contamination. For instance, DnBP was more present in bread bought from supermarket N than in bread from other bakeries, BBP levels in bread samples from fresh bakery F and supermarket N were remarkably higher than BBP concentrations in other bread samples and the measured DEHP concentration ranges were higher in the bread samples from fresh bakery A and supermarket J compared to those from the other locations.

Table 22: Concentrations of DiBP, DnBP, BBP and DEHP (min-max (median); in µg/kg fresh weight) in 43 purchased bread samples grouped by flour type, packaging type, location, form and contact time with bread bag.

	N	DiBP	DnBP	BBP	DEHP
Total	43	1.1-74 (2.9)	ND-106 (3.7)	ND-6.9 (0.7)	ND-2264 (63)
Flour type					
White	21	1.3-34 (2.7)	ND-42 (3.2)	ND-6.9 (0.8)	ND-1002 (63)
Brown/wholemeal	22	1.1-74 (2.9)	ND-106 (4.1)	ND-5.9 (0.8)	ND-2264 (60)
Packaging type					
Paper	32	1.1-74 (3.2)	ND-106 (3.2)	ND-6.9 (0.7)	ND-2264 (56)
Paper with plastic window	5	1.3-2.7 (1.8)	1.7-4.4 (3.7)	ND-0.6 (ND)	ND-343 (206)
Plastic	6	2.1-6.8 (3.3)	ND-19 (4.4)	0.1-1.8 (1.0)	ND-361 (ND)
Location					
Fresh bakery	*20*	*1.4-74 (4.2)*	*ND-47 (3.1)*	*ND-6.9 (0.7)*	*ND-2264 (56)*
Location A	4	1.4-74 (18)	ND-15 (ND)	ND-0.1 (ND)	417-2264 (1012)
Location B	2	4.5-4.6 (4.6)	ND-5.0 (ND)	0.4-0.7 (0.6)	88-312 (200)
Location C	2	21-39 (30)	2.3-2.7 (2.5)	0.7-2.5 (1.6)	ND
Location D	2	2.7-2.9 (2.8)	9.6-16 (13)	0.8-1.1 (0.9)	282-392 (337)
Location E	2	3.1-4.0 (3.6)	5.1-12 (9.0)	0.9-1.3 (1.1)	38-63 (51)
Location F	2	2.5-2.8 (2.7)	2.3-2.9 (2.6)	5.9-6.9 (6.4)	49-74 (62)
Location G	2	6.2-6.7 (6.5)	3.2-4.2 (3.7)	0.7-0.8 (0.8)	42-45 (44)
Location H	2	4.4-6.0 (5.2)	8.5-47 (28)	0.5-1.7 (1.1)	ND-34 (17)
Location I	2	1.8-1.9 (1.9)	ND	ND-0.4 (ND)	ND-47 (24)
Supermarket	*23*	*ND-17 (2.6)*	*ND-106 (4.0)*	*ND-4.6 (0.5)*	*ND-1073 (67)*
Location J	2	11-17 (14)	ND-2.5 (1.3)	0.8-1.5 (1.2)	1002-1073 (1038)
Location K	2	2.7-3.7 (3.2)	ND-2.6 (1.3)	0.6-1.1 (0.9)	245-361 (303)
Location L	1	2.9	ND	1.5	268
Location M	2	1.3-2.0 (1.7)	3.7-4.0 (3.9)	ND	ND-343 (172)
Location N	2	1.7-1.9 (1.8)	42-106 (74)	4.4-4.6 (4.5)	ND-67 (34)
Location O	2	2.2-2.3 (2.3)	3.2-4.5 (1.9)	ND-1.3 (0.7)	33-198 (116)
Location P	2	1.3-1.8 (1.6)	1.7-4.4 (3.1)	ND	ND-206 (103)
Location Q	2	1.1-1.5 (1.3)	2.0-2.8 (2.4)	ND-0.8 (0.4)	123-148 (136)
Location R	2	5.8-6.8 (6.3)	4.0-4.8 (4.4)	0.8-1.8 (1.3)	ND
Location S	2	6.5-7.2 (6.9)	ND-7.6 (3.6)	ND-0.2 (0.1)	22-28 (25)
Location T	2	2.1-2.8 (2.5)	18-19 (19)	0.1-0.5 (0.3)	ND-80 (40)
Location U	2	2.6-3.3 (3.0)	13-19 (16)	0.3-0.4 (0.4)	ND

	N	DiBP	DnBP	BBP	DEHP
Form					
Long	31	1.1-39 (2.7)	ND-19 (3.5)	ND-6.9 (0.7)	ND-2264 (69)
Round	12	1.7-74 (6.4)	ND-106 (3.4)	ND-4.6 (0.6)	ND-1073 (156)
Contact time with bread bag					
Long (prepacked)	7	2.1-6.8 (2.9)	ND-19 (4.0)	0.1-1.8 (0.8)	ND-361 (80)
Short (packed at purchase)	36	1.1-74 (2.9)	ND-106 (3.5)	ND-6.9 (0.6)	ND-2264 (56)

N: Number of samples; ND: not detected; LODs: 0.1 µg/kg fresh weight (BBP) and 1 µg/kg fresh weight (DiBP, DnBP and DEHP).

DiBP, DnBP and DEHP levels of the paper bag of the two high DEHP containing bread samples amounted to 1.4, 4.2 and 33 ng/cm², respectively; BBP could not be detected. The corresponding absolute phthalate contents amounted to 1.7 µg DiBP, 5.0 µg DnBP and 40 µg DEHP per bread bag. For comparison, absolute phthalate contents in the two investigated bread samples amounted to 14 and 6.6 µg for DiBP, 2.0 and <1.2 µg for DnBP, 0.6 and 0.9 µg for BBP and 802 and 644 µg for DEHP.

The homemade "all-in" bread sample absolutely contained 34 µg DiBP, 1.6 µg DnBP, 0.6 µg BBP and 113 µg DEHP. The all-in bread mix used to bake this bread absolutely contained DiBP, DnBP, BBP and DEHP in concentrations of 3.0, <0.5, <0.3 and <1.5 µg, respectively. The absolute phthalate levels in the wholemeal homemade bread sample amounted to 15 µg for DiBP, <2.4 µg for DnBP, 1.8 µg for BBP and 39 µg for DEHP compared to 3.8, 0.8, <0.3 and 8 µg in the wholemeal flour mix, respectively. Last, the dark homemade bread absolutely contained 697 µg DiBP, 6.5 µg DnBP, 0.6 µg BBP and 41 µg DEHP. Absolute concentrations in the corresponding Black Forrest flour mix amounted to 692 µg DiBP, 8.5 µg DnBP, 0.4 µg BBP and 3.1 µg DEHP.

II.2.3.3 Concentration profiles

Table 23 gives an overview of the concentrations of the most prominent phthalates (DiBP, DnBP, BBP and DEHP) in the concentration profiles of apple, bread, soft goat's cheese, salami and semi-soft cheese. In the concentration profile of apple, the surface (skin) sample contained significantly more DiBP, DnBP, BBP and DEHP than the core (flesh) sample. Concentrations in the skin amounted to 480, 18, 58 and 104 µg/kg fresh weight, respectively, while the flesh only contained DEHP at a detectable level of 2.6 µg/kg fresh weight. In the investigated bread sample on the other hand, concentrations of the four phthalates were nearly identical in crust and crumbs, especially considering the fact that phthalate concentrations in the crust are more concentrated compared to phthalate levels in the crumbs as a result of moisture loss during the baking process. Also in the investigated soft goat's cheese and semi-soft cheese samples, phthalates were uniformly distributed (i.e. after taking into account a measurement uncertainty of 30% (see Section II.2.2.2)). In salami, DEHP concentrations were significantly higher within the salami (131-180 µg/kg fresh weight) than in the rind (26 µg/kg fresh weight), whereas the three other phthalates were mostly found in the rind.

Table 23: Concentrations of DiBP, DnBP, BBP and DEHP (in µg/kg fresh weight) analysed in the concentration profiles of apple, bread, soft goat's cheese (GC), salami (S) and semi-soft cheese (C).

	DiBP	DnBP	BBP	DEHP
Apple				
Surface (skin)	480	18	58	104
Core (flesh)	ND	ND	ND	2.6
Bread				
Surface (crust)	19	3.9	ND	286
Core (crumbs)	2.2	ND	0.3	226
Soft goat cheese				
GC1 (surface - top)	9.1	ND	0.5	546
GC2 (core)	8.5	ND	ND	507
GC3 (surface - bottom)	7.8	ND	0.2	430
GC4 (surface - side)	8.3	ND	0.4	499
Salami				
S1 (rind)	36	15	2.3	26
S2 (surface)	6.3	11	ND	131
S3 (between surface and core)	6.3	9.3	ND	180
S4 (core)	4.3	6.4	ND	177
Semi-soft cheese				
C1 (middle - top)	8.5	7.1	ND	238
C2 (middle - core)	4.8	4.5	ND	186
C3 (middle - bottom)	8.9	6.8	ND	289
C4 (left - top)	9.5	7.3	6.1	209
C5 (left - core)	10	9.4	ND	258
C6 (left - bottom)	14	11	10	328

ND: not detected.

II.2.4 Discussion

II.2.4.1 General analytical results

In general, median phthalate concentrations of the different groups investigated during MC2 were similar to the concentrations determined in MC1 of the PHTAL project. Therefore, literature discussed in Fierens et al. (2012c) will not be repeated here and the phthalate concentrations measured will only be compared with studies published recently (i.e. from 2012 onwards).

Guo et al. (2012) analysed nine phthalates in eight categories of Chinese foodstuffs. DMP, DEP, DBP, DiBP, BBP and DEHP were frequently detected. DEHP was the major phthalate found in most of the samples with concentrations ranging from below the LOQ to 762 µg/kg. DMP was found in 82% of the food samples analysed, which is different from the samples analysed in the PHTAL project, where DMP was rarely detected (Fierens et al., 2012c).

Schecter et al. (2013) reported concentrations of nine phthalates in 72 individual American food samples purchased in New York State. Also in this study, DEHP was the most detected phthalate compound. Median phthalate concentrations in beverages, milk, fish, grain, meat and meat products, condiments and infant food were generally comparable to the ones measured in the PHTAL project. Lower median concentrations were measured by Schecter et al. (2013) for DEHP in fruit and

vegetables (1.85 µg/kg compared to 16 µg/kg in the PHTAL project) and for BBP in vegetable oils (2.2 µg/kg compared to 29 µg/kg in the PHTAL project).

Bradley et al. (2013a) tested 261 British foodstuffs for their phthalate levels. Phthalates were confirmed to be present in 77 samples and the highest incidence was for DEHP (66 samples) with the highest concentration being detected in an oil sample (6447 µg/kg). Of the 261 foodstuffs tested, the highest concentration was determined for DiNP in a fish sample (> 11,000 µg/kg). The DEP, DiBP, DnBP, BBP and DEHP concentration ranges reported by Bradley et al. (2013a) are comparable to those determined in the PHTAL project. DMP, DCHP and DnOP were reported not to be present in the British foodstuffs, although responses for some analytes were observed by Bradley et al. (2013a), but without meeting the confirmation criteria.

II.2.4.2 Bread

Of all investigated parameters, only the location affected phthalate contamination in bread as remarkable higher phthalate concentration ranges were detected at some of the investigated locations compared to others (Table 22). Bradley et al. (2007) among others observed that phthalates can be present in coatings of non-stick cookware products most likely due to the coatings picking up these substances when the cookware products are packaged (in printed boxes or sleeves made of cardboard) and transported. So, differences regarding the use of other types of baking equipment (e.g. kneading machines and baking trays) between the investigated bakeries may have influenced the occurrence of phthalates in bread.

Besides the use of other baking equipment types, the use of other ingredients and packaging materials might also explain the dissimilarities noticed between the investigated bakeries. The role of the packaging material on phthalate contamination in bread was further explored in this study by comparing absolute contents of phthalates in two supermarket bread samples with absolute phthalate levels measured in the paper bag in which they were packed. In this study, DnBP most likely migrated from the packaging into bread, since absolute DnBP contents in the paper bag (5.0 µg) were higher than in the two investigated bread samples (<1.2 – 2.0 µg). However, the paper bag could not be the most important contamination source of DiBP, BBP and DEHP in these breads. Even if 100% migration would be assumed, only 1.9 µg of DiBP and 40 µg of DEHP could have been derived from the paper bag, which is far below the absolute DiBP (6.6-14 µg) and DEHP (644-802) levels that were analysed in the two bread samples.

Whether bread is contaminated with phthalates due to the use of phthalate containing ingredients was examined by comparing absolute phthalate concentrations in homemade bread with phthalate concentrations detected in their most important ingredient (i.e. flour). In the dark homemade bread sample, the Black Forest flour mix used was most likely responsible for DiBP, DnBP and to a smaller degree also BBP contamination, since absolute contents of these phthalates in the flour mix were nearly identical to the concentrations measured in the dark bread sample. However, this Black Forest flour mix turned out not to be the most important contamination source for DEHP in the dark homemade bread.

Considering all these observations, it can be concluded that phthalates are not present in Belgian bread because of one sole source, but rather due to a combination of sources. The largest part of the contamination takes place during processing – either by the use of contaminated ingredients or by

the use of phthalate containing baking equipment – meaning that phthalate contamination in Belgian bread is mainly bakery dependent. The role of the packaging material on phthalate contamination is of less importance.

II.2.4.3 Concentration profiles

Phthalates were uniformly distributed within the investigated bread, soft goat's cheese, salami and semi-soft cheese samples, but not in apple (Table 23). Unlike apple, all these food products have in common that they are processed in the same manner. This is strong evidence that food preparation (i.e. baking, mixing of ingredients, pasteurisation, and so on) is introducing phthalates in Belgian food products rather than packaging followed by migration.

The possibility that processing is the most important contamination pathway is strengthened by the observation in salami. DEHP, a high molecular mass phthalate, was found 5-7 times more within the salami than in the rind. DiBP, DnBP and BBP on the other hand, which all have a lower molecular mass than DEHP, were found mostly in the rind of the salami (Table 23). The higher the molecular mass of a chemical compound, the less it will migrate (Castle, 2007; Hansen et al., 2013) . If salami was contaminated with phthalates via migration from the rind to the interior of the salami, than DEHP would show little penetration into the interior part rather than the most interior penetration of all phthalates.

In the investigated apple sample, skin contained more DiBP, DnBP, BBP and DEHP than flesh (Table 23). A similar observation was done by Jiang et al. (2005) for DnOP in apples. Unlike for the processed foods, here, external sources have to be responsible for introducing phthalates in apple. Possible sources might be the use of phthalates in packaging materials, pesticides and/or the use of phthalates in wax fruit coatings (Cao, 2010; Lin and Zhao, 2007; Navarro-Tarazaga et al., 2008; Yin et al., 2011). However, in this study, packaging can be excluded as contamination source for apple considering the investigated apple sample was not packed at purchase.

Bradley et al. (2013a) conducted a concentration profile of lasagne sheets packed in cardboard in order to determine contamination sources for DiBP. The highest DiBP concentration was found in the lasagne sheet that had come into direct contact with the cardboard packaging, which provided evidence that the lasagne sheet was contaminated with DiBP via the recycled board packaging.

II.2.4.4 Recommendations for future research

Investigating potential contamination sources for phthalates in food products has been a topic of interest for many decades (Cao, 2010). This study certainly contributed to this subject by revealing that processing – rather than packaging – might be the most important contamination source for phthalates in food products available on the Belgian market. Nevertheless, the authors would like to do some recommendations for future research regarding the investigation of phthalate contamination in bread. Within the PHTAL project, 43 purchased bread samples were examined. The analytical results indicated that bread contamination in Belgium – and thus maybe also in other countries – is bakery dependent. Because the presence of several potential confounding factors (e.g. packaging time, contact time and flour type), this dependency could regrettably not statistically be confirmed, but could be done in another study by measuring, e.g. phthalate contents in several samples of white, round breads packed at purchase in paper bags deriving from multiple bakeries. Furthermore, at one or more bakeries, samples could be collected and analysed at several stages

during the production process of bread. For instance, phthalates could be analysed in the ingredients, in the dough before and after rising and in the bread after baking, slicing and packing.

II.2.5 Conclusions

During the PHTAL project, levels of eight phthalates were measured in 591different foods and 30 different packaging materials in order to determine contamination pathways for phthalates in food products present on the Belgian market. For the four most abundant phthalates (DiBP, DnBP, BBP and DEHP), two topics were investigated in more detail: concentrations in bread and concentration profiles in different food items. The analytical results of both items revealed that the preparation of processed foods, and not packaging, is the most important source of phthalate contamination in Belgian food products.

Supplementary data

Supplementary data can be found in Table 54 (Annexes).

II.3 PHTAL 3 – Measuring the dietary exposure to phthalates in the Belgian adult population

Sioen, I., Fierens, T., Van Holderbeke, M., Geerts, L., Bellemans, M., De Maeyer, M., Servaes, K., Vanermen, G., Boon, P.E. and De Henauw, S. (2012). ***Phthalates dietary exposure and food sources for Belgian preschool children and adults.*** *Environment International, 48, 102-108.*

Abstract

Numerous studies have indicated that for many phthalates, the intake of contaminated foods is the most important exposure pathway for the general population. Up to now, data on dietary phthalate intake are scarce and – to the authors' knowledge – not available for the Belgian population. Therefore, the purpose of this study was: (1) to assess the long-term intake of the Belgian population for eight phthalates considering different exposure scenarios (benzylbutyl phthalate (BBP); di-*n*-butyl phthalate (DnBP); dicyclohexyl phthalate (DCHP); di(2-ethylhexyl) phthalate (DEHP); diethyl phthalate (DEP); diisobutyl phthalate (DiBP); dimethyl phthalate (DMP), di-*n*-octyl phthalate (DnOP)); (2) to evaluate the intake of BBP, DnBP, DEP and DEHP against tolerable daily intake (TDI) values; and (3) to assess the contribution of the different food groups to the phthalate intake. The intake assessment was performed using a Belgian food consumption database of adults (≥ 15 years old), combined with a database of phthalate concentrations measured in over 550 food products sold on the Belgian market. Phthalate intake was calculated using the "Monte Carlo Risk Assessment" programme (MCRA 7.0). The intake of DEHP was the highest, followed by DiBP. The intake of BBP, DnBP and DEP was far below the TDI. Bread was the most important contributor to the DEHP intake and this may deserve further exploration, since the origin of this phthalate in bread remains unclear.

II.3.1 Introduction

Phthalates (dialkyl or alkyl aryl esters of *o*-phthalic acid) are a group of organic chemicals that have been used in a large variety of industrial and consumer applications for more than 50 years. They are the most commonly used plasticizers worldwide (MAFF UK, 1996; Wittassek et al., 2011). Depending on the alcohol that makes up the alkyl chain, phthalates have a wide range of different properties for divers applications, including cosmetics, floorings, pharmaceutical products, medical devices, toys, food contact and packaging materials, inks and glues (Fromme et al., 2007b; Wittassek et al., 2011). Phthalates are released into the environment and into food items by direct release, migration, evaporation, leaching and abrasion of and from the products they are used in (Wittassek et al., 2011). Consequently, phthalates contaminate the indoor environments and the human food chain and belong to the ubiquitous environmental contaminants today (Fromme et al., 2007b; Wormuth et al., 2006). As a result, the general population is widely and continuously exposed to phthalates.

During the last ten years, phthalates have attracted the public attention due to possible harmful effects to both the environment (Bradlee and Thomas, 2003; Parkerton and Staples, 2003) and human health (Hauser and Calafat, 2005; Kamrin, 2009; Meeker et al., 2009; Shea, 2003). Phthalates have toxic effects on liver, kidney and the reproductive system and/or can act as endocrine disrupting agents. Numerous studies have indicated that for phthalates, and especially for di(2-ethylhexyl) phthalate (DEHP), the intake of contaminated foods is the most important exposure pathway for the general population (Wittassek et al., 2011; Wormuth et al., 2006). Due to their possible harmful effects and the importance of dietary intake as exposure route, tolerable daily

intakes (TDI) have been specified by the European Food Safety Authority (EFSA) for several phthalates, namely 10 µg/kg bw/day for di-*n*-butyl phthalate (DnBP) (EFSA, 2005a), 50 µg/kg bw/day for DEHP (EFSA, 2005c), 500 µg/kg bw/day for benzylbutyl phthalate (BBP) (EFSA, 2005b), and a TDI of 150 µg/kg bw/day for diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) (EFSA, 2005d; 2005e). Besides, the World Health Organisation (WHO) defined a TDI for diethyl phthalate (DEP) of 5,000 µg/kg bw/day (WHO, 2003). Risk characterisation for these phthalates can be conducted by evaluating the assessed dietary intake against these TDI values. However, up to now, data on dietary phthalate intake are scarce and – to the authors' knowledge - not available for the Belgian population. Therefore, more phthalate intake data are needed.

Between 2009 and 2011 - by order of the Belgian Federal Public Service of Health, Food Chain Safety and Environment - a research project (acronym: PHTAL) was conducted with three main objectives:

- To obtain accurate and sensitive data of phthalates in all kinds of food products and packaging materials sold on the Belgian market (results of this first part are recently published (Fierens et al., 2012c)) ;
- To gain a clear understanding of possible contamination pathways for phthalates in the Belgian food market;
- To estimate dietary exposure to phthalates in the Belgian population.

In this manuscript, the results of the third research objective are described, i.e. (1) the intake assessment of Belgian adults for eight phthalates (BBP; DnBP; dicyclohexyl phthalate (DCHP); DEHP; DEP; diisobutyl phthalate (DiBP); dimethyl phthalate (DMP), di-*n*- octyl phthalate (DnOP)); (2) the intake evaluation of BBP, DnBP, DEP and DEHP against the TDI values; and (3) the contribution assessment of the different food groups to the intake of the eight phthalates.

II.3.2 Materials

II.3.2.1 Food consumption data

The food consumption database used in this study contains the results of the first Belgian national food consumption survey. In this survey, food consumption data were collected for a representative sample of the Belgian adult population from 15 years old onwards (minimum, median and maximum age: 15, 57 and 98 years, respectively). The study design followed the recommendations of the European Food Consumption Survey Method project (Brussaard et al., 2002a; Brussaard et al., 2002b; De Henauw et al., 2002). The food consumption data were collected during 2004 by means of a repeated (two times) non-consecutive 24h recall (face to face). The one year survey was distributed equally over the seasons and days of the week. During the 24h recall, the participants reported types and quantities of all foods and beverages that were consumed the preceding day. The 24h recall was carried out using the validated EPIC-SOFT program to obtain a standardised interview (Slimani and Valsta, 2002). The software allowed obtaining a very detailed description and quantification of foods and recipes consumed. Quantification of the consumed foods was supported by a picture book that compromises photographs of foods in different portion sizes. Complete data were available for 3,083 adults: 1,523 women and 1,538 men (for 22 adults, the gender was not reported). The Belgian national food consumption survey was approved by the Ethical Committee of the Scientific Institute of Public Health (Brussels, Belgium). More details about the methodology and the population

characteristics of this survey are reported earlier elsewhere (De Vriese et al., 2005; Temme et al., 2010).

II.3.2.2 Phthalate concentration data and analytical procedure

For the intake assessment, concentration data of eight phthalates – BBP, DnBP, DCHP, DEHP, DEP, DiBP, DMP and DnOP – in food products sold on the Belgian market between 2009 and 2011 were used. These data resulted from the first part of the PHTAL project, aiming to obtain accurate and sensitive data of phthalates in all kinds of food products sold on the Belgian market. The phthalate concentration database was set up based on three measurement campaigns. In the first campaign, 388 food products were selected based on (1) consumption data from the most recent Belgian national food consumption survey (De Vriese et al., 2005) and (2) the likelihood that foodstuffs contain phthalates. The selection of the foods for the second campaign was based on the results of the first one: 206 extra samples were bought from foods in which high phthalate concentrations were found in the first campaign. In the third campaign, frequently consumed foods (n=80) which are usually not eaten without preparation (e.g. potatoes, pasta, rice, fresh meat) were analysed raw as well as prepared (boiled, steamed, (deep-)fried or grilled) to assess the effect of the preparation on the phthalate concentration. The results of the third measurement campaign are described (Fierens et al., 2012a), but in short it can be stated that preparation generally caused a decline in phthalate concentrations.

The analytical method used to measure the phthalate concentrations in the food samples of the three different campaigns is described in detail by Fierens et al. (2012b; 2012c) illustrated with specific results. In short, for the sample preparation, a distinction was made between high-fat foods, low-fat foods and aqueous-based beverages. Briefly, 5 to 20 g of every high-fat food sample was extracted to obtain at least 0.5 g fat. For the extraction of low-fat foods, 10 g was used. For aqueous-based beverages, a liquid–liquid extraction was applied using 500 ml of each sample. Subsequently, all extracts were purified via gel permeation chromatography and the phthalates of interest were analysed by means of gas chromatography-low resolution-mass spectrometry with electron impact ionisation. Detection of the different phthalate compounds took place in selected ion monitoring mode. For all phthalates except DMP, the dominant product ion *m/z* 149 was used as target ion; for DMP, *m/z* 163 was used. As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compounds in a sample had to be within 20% of that observed in a standard solution. Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (twelve samples at the most) to reduce the risk of contamination (Fierens et al., 2012b; 2012c).

Because phthalates are ubiquitous, limits of quantification (LOQs) strongly depended on the feasible blank concentrations. Therefore, for each phthalate compound, LOQ calculations were based on the concentrations detected in the procedural blanks – i.e. the sum of the average blank concentration and six times the standard deviation of replicate procedural blank measurements. In the three measurement campaigns, LOQs varied between 5 and 145 µg/kg fat (0.3 and 145 µg/kg product, respectively) for high-fat food samples, between 0.2 and 8.0 µg/kg product for low-fat food samples and between 0.01 and 0.03 µg/kg product for aqueous-based beverages.

The food consumption survey used did not contain all the food items that were analysed in the three measurement campaigns (i.e. some food products were relatively new on the market or some foodstuffs were stored in the food consumption database as their ingredients instead of the whole product; e.g. ready to eat meals). In total, phthalate concentrations of 356, 141 and 75 food products from the first, second and third measurement campaigns, respectively, were used for the intake assessment.

II.3.2.3 Linking food consumption data and phthalate concentration data

To link the food consumption database with the phthalate concentration database, 90 different food groups were created, based on their nutritional composition and phthalate concentrations (Table 24). For some food items analysed in the PHTAL project, the concentrations of one or more phthalates could not be quantified because of interference in the chromatogram peaks, resulting in missing values. When this resulted in absence of concentration values for specific phthalates in an overall food group, the missing values were replaced by the highest concentration (worst case) of a related food group. For example, DnBP concentrations for coffee and tea were missing and therefore replaced by the highest DnBP concentration analysed in water. Detailed quantitative data of the phthalate concentrations in the different food groups are recently published by Fierens et al. (2012c).

Table 24: Overview of the 90 different food groups created to link the food consumption database with the phthalate concentration database.

	Food group	Number of samples[1]
1	Potatoes	12 (7)
2	Deep-fried potato products	12 (6)
3	Vegetables and pulses	40
4	Fruits	12
5	Olives	1
6	Nuts and seeds	4
7	Walnuts	1
8	Milk (beverages)	16
9	Buttermilk	3
10	Cottage cheese	1
11	Low-fat yoghurt	1
12	High-fat yoghurt	4
13	Low-fat curd cheese	2
14	High-fat curd cheese	1
15	Cheese	21
16	Feta cheese	3
17	Goat's cheese	5
18	Puddings and milk desserts	7
19	Cream	5
20	Coffee cream (liquid)	1
21	Flour	3
22	Binding agents	2
23	Oatmeal	3
24	Pasta and other grains	34 (9)
25	Rice	28 (9)
26	Ravioli	1
27	Bread	51
28	Rusks and crackers	4
29	Coffee cream (powder)	1
30	Breakfast cereals	7
31	Crisps and salty biscuits	3
32	Prawn crackers	1
33	Frying fat	5

	Food group	Number of samples[1]
34	Popcorn	3
35	Dough (pizza, puff, crumble)	2
36	Fresh meat	25
37	Poultry	2
38	Processed meat	23
39	Pesto, green	2
40	Pesto, red	1
41	Eggs	2
42	Olive oil	5
43	Margarine	9
44	Butter	3
45	Tomato sauce	4
46	Dressing and vinaigrette	4
47	Sugar, sweeteners, syrups	1
48	Jam, marmelade and honey	2
49	Chocolate spread	1
50	Cocoa powder	2
51	Chocolate and candy bars	2
52	Chewing gum	2
53	Confectionery (excl. chocolate)	2
54	Syrup Liège	1
55	Ice cream	1
56	Biscuits and cakes	14
57	Fruit and vegetable juice, sorbet and water ice	22
58	Soft drinks (incl. non-alcoholic beers)	25
59	Coffee and tea	2
60	Water	19
61	Alcoholic drinks	20
62	Sauces	6
63	Mayonnaise	11
64	Soup and stock	5
65	Meat replacements (not soya)	1
66	Soya cream, milk and dessert	2
67	Tofu, tempeh, miso	2
68	Meal replacement	1
69	Vinegar	1
70	Mustard	2
71	Salt and pepper	1
72	Stock cube	1
73	Spring roll	1
74	Deep-fried snacks	1
75	Aiki noodles	1
76	Deep-fried meat products	1
77	Fish products	1
78	Fish products covered in breadcrumbs	1
79	Herring and rollmop	2
80	Shellfish	3
81	Low-fat fish	8
82	Smoked fish	2
83	High-fat fish	9
84	Grape seed oil	3
85	Salad oil	4
86	Coleseed oil	1
87	Corn oil	2
88	Sunflower oil	2
89	Peanut oil	1
90	Yeast and gelatine	1

[1] A number between parentheses represents the number of processed food samples within a food group.

II.3.2.4 Assessing the intake distributions

The long-term dietary exposure to phthalates was calculated using the "Monte Carlo risk assessment" programme (MCRA), release 7.0, available for registered users at the RIVM website (https://mcra.rivm.nl). For the estimation of the exposure, daily consumption patterns (e.g. 6,166 measurements, 2 days × 3,083 Belgian adults) were multiplied with the food group-specific phthalate concentrations, and summed over all foods consumed per day per individual. In this way, the whole diet was addressed when assessing the exposure to phthalates. The estimated exposures were adjusted for the individuals' body weight.

The resulting distribution of daily phthalate exposures includes both the variation between individuals and between days within individuals. However, to assess the long-term intake within a population only the former type of variation is of interest, since in the long run the intake between different days of one individual will level out. Therefore, the distributions of daily exposures were corrected for the within-person (between days) variation using the betabinomial-normal model (BBN) (Slob, 2006). For this the positive daily exposure distribution was logarithmically transformed in a normal distribution, an important prerequisite to use the BBN model for estimating long-term exposure which should be checked. We did this by using the normal quantile-quantile (q-q) plot (Boon et al., 2011; De Boer et al., 2009). In this paper we report the 50^{th}(P50), 95^{th} (P95) and 99^{th} percentiles (P99) of the corrected, long-term intake distributions of the eight phthalates, since the interest goes to the higher end of the intake distribution. Moreover, for each phthalate, we report the contribution (in percentage) of the three most important food groups to the intake of a certain phthalate.

The intake of each phthalate was calculated for 12 different exposure scenarios:

(1) two scenarios using the overall distribution of the concentrations, differing in the fact that in the first one the effect of preparation was not considered (only concentrations measured in unprepared foods were used), while in the second one the effect of preparation was considered [2 scenarios];

(2) on top of that, two other scenarios were applied using the overall distribution of concentrations per food group (probabilistic approach) versus only the maximum phthalate concentration per food group (worst case semi-probabilistic approach) [2 x 2 scenarios = 4 scenarios];

(3) on top of that, three scenarios were conducted related to the concentrations assigned to food samples with a phthalate concentration below the LOQ, i.e. the lower bound (LB), medium bound (MB) and upper bound (UB) approach, meaning that concentrations assigned were equal to 0 mg/kg, ½ LOQ and LOQ, respectively [4 x 3 scenarios = 12 scenarios].

The reason why such a worst case scenario was considered in this study is that for some food items it can happen that people always buy the same food in the same shop and that there is a source of contamination in that specific shop/production place, e.g. for bread: some people always buy the same bread in the same bakery.

II.3.3 Results

Table 25 shows the 50th, 95th and 99th percentiles of the intake distributions for the eight phthalates, for Belgian adults. For each phthalate, the results of all 12 scenarios are given. For Belgian adults, the intake of DEHP was the highest, followed by DiBP and DnBP. Medium intakes were found for BBP and DEP, and the lowest intakes were found for DCHP, DMP and DnOP. In general, the intakes assessed in the worst case scenarios (using only the maximum phthalate concentration per food group) were five to ten times higher compared to the scenario that considered all measured concentrations within a food group (probabilistic approach). Accounting for the effect of processing on phthalate concentrations resulted only in a negligible decline in the assessed intakes, except for DiBP and DEHP (Table 25). The influence of replacing concentration below the LOQ on the assessed intakes differed between phthalates. The effect was negligible for phthalates with a high intake (DEHP, DnBP and DiBP), but rather high (increase with factor three to four) for phthalates with low assessed intakes (e.g. DnOP and DMP) (Table 25).

Performing a risk characterisation by comparing the intakes of BBP, DnBP, DEP and DEHP with their respective TDI values showed that for the Belgian adults no health risks were to be expected for the dietary intake of BBP, DnBP and DEP, since the intakes were far below the TDI of 500, 10 and 5,000 µg/kg bw/day, respectively (EFSA, 2005a; 2005b; WHO, 2003).

Table 25: Percentile 50th (P50), 95th (P95) and 99th (P99) of the long-term intake distribution of the eight considered phthalates for adults, considering 12 exposure scenarios per phthalate.

	TDI value		Probabilistic scenario[a]			Probabilistic scenario considering preparation[b]			Worst case scenario[c]			Worst case scenario considering preparation[d]		
			P50	P95	P99	P50	P95	P99	P50	P95	P99	P50	P95	P99
			Intake in µg/kg bw/day											
BBP	500	LB	0.050	0.111	0.153	0.049	0.109	0.152	0.240	0.539	0.738	0.235	0.532	0.730
		MB	0.055	0.118	0.157	0.054	0.117	0.158	0.240	0.540	0.740	0.235	0.533	0.732
		UB	0.060	0.125	0.168	0.060	0.124	0.167	0.240	0.541	0.742	0.235	0.534	0.734
DnBP	10	LB	0.073	0.142	0.184	0.069	0.134	0.177	0.585	1.23	1.64	0.563	1.20	1.61
		MB	0.085	0.162	0.211	0.081	0.155	0.201	0.590	1.24	1.65	0.568	1.21	1.62
		UB	0.096	0.183	0.239	0.093	0.175	0.227	0.595	1.25	1.66	0.574	1.22	1.64
DEHP	50	LB	1.57	2.99	3.87	1.47	2.79	3.66	8.01	15.4	19.8	7.15	14.0	18.0
		MB	1.59	3.02	3.93	1.49	2.86	3.69	8.01	15.4	19.8	7.16	14.0	18.0
		UB	1.62	3.09	3.99	1.52	2.92	3.79	8.01	15.4	19.8	7.16	14.0	18.0
DCHP	NA	LB	0.011	0.031	0.046	0.011	0.031	0.045	0.072	0.149	0.196	0.072	0.148	0.194
		MB	0.019	0.042	0.059	0.019	0.042	0.059	0.076	0.156	0.205	0.076	0.156	0.206
		UB	0.026	0.055	0.075	0.026	0.055	0.075	0.080	0.163	0.214	0.081	0.164	0.216
DEP	5,000	LB	0.030	0.066	0.091	0.025	0.052	0.070	0.304	0.765	1.121	0.152	0.317	0.424
		MB	0.046	0.090	0.119	0.041	0.079	0.105	0.313	0.775	1.128	0.163	0.335	0.448
		UB	0.062	0.119	0.156	0.057	0.108	0.140	0.322	0.788	1.125	0.177	0.358	0.478
DiBP	NA	LB	0.168	0.348	0.468	0.136	0.269	0.351	2.28	4.70	6.17	1.95	4.02	5.21
		MB	0.174	0.357	0.481	0.143	0.280	0.370	2.28	4.70	6.17	1.95	4.02	5.21
		UB	0.180	0.365	0.487	0.149	0.290	0.382	2.28	4.71	6.18	1.96	4.02	5.21

	TDI value		Probabilistic scenario[a]			Probabilistic scenario considering preparation[b]			Worst case scenario[c]			Worst case scenario considering preparation[d]		
			P50	**P95**	**P99**	**P50**	**P95**	**P99**	**P50**	**P95**	**P99**	**P50**	**P95**	**P99**
							Intake in µg/kg bw/day							
DMP	NA	LB	0.011	0.021	0.027	0.010	0.021	0.027	0.068	0.131	0.167	0.066	0.127	0.162
		MB	0.014	0.027	0.035	0.014	0.027	0.035	0.070	0.135	0.173	0.068	0.133	0.170
		UB	0.018	0.034	0.043	0.018	0.033	0.043	0.072	0.139	0.179	0.071	0.138	0.178
DnOP	NA	LB	0.005	0.010	0.014	0.005	0.009	0.013	0.056	0.119	0.160	0.051	0.112	0.153
		MB	0.015	0.030	0.039	0.014	0.029	0.038	0.062	0.130	0.177	0.059	0.125	0.171
		UB	0.024	0.049	0.089	0.024	0.049	0.066	0.067	0.140	0.190	0.066	0.139	0.188

LB: lower bound, MB: medium bound, NA: not available, TDI: tolerable daily intake; UB: upper bound; [a] using all measured concentrations; [b] using all measured concentrations considering the effect of processing for some food groups; [c] using the maximum concentration per food group; [d] using the maximum concentration per food groups considering the effect of processing for some food groups.

Table 26 shows for each phthalate the top three of the food groups contributing most to the dietary phthalate intake in Belgian adults. The reported contributions are based on the results of the medium bound probabilistic scenario taking into account the effect of preparation, since this is assumed to be the most realistic exposure scenario. The percentage contributions show that – depending on the phthalate - the top three of food groups contributed between 42 and 66% to the total dietary intake of the phthalate in adults. Bread was an important contributor for several phthalates and in particular for DnBP, DEHP and DiBP for which the highest intakes were found. Other important contributors for different phthalates were fruits, fresh & processed meat and biscuits & cakes, indicating that these food groups contain a mixture of different phthalates. All these food groups are frequently consumed in high amounts by the Belgian population. Milk & milk beverages as well as cheese contributed only considerably to the intake of DCHP and olive oil was an important contributor to the intake of BBP in adults.

Table 26: Top three of the food groups contributing most to the long-term intake of the eight phthalates by Belgian adults. Results from the medium bound probabilistic scenario taking into account the effect of preparation are listed.

	First	Second	Third	% contribution of top 3
BBP	Fruits (28.3%)	Olive oil (14.9%)	Alcoholic drinks (14.0%)	57.2
DnBP	Bread (19.7%)	Processed meat (14.0%)	Biscuits and cakes (10.7%)	44.4
DEHP	Bread (31.4%)	Fruits (8.6%)	Fresh meat (8.1%)	48.1
DCHP	Cheese (21.5%)	Milk (beverages) (11.3%)	Biscuits and cakes (9.5%)	42.3
DEP	Fruits (20.9%)	Bread (11.8%)	Pasta and other grains (10.0%)	42.7
DiBP	Bread (33.5%)	Fruits (17.3%)	Biscuits and cakes (14.9%)	65.7
DMP	Fresh meat (27.2%)	Processed meat (14.6%)	Vegetables and pulses (7.2%)	49.0
DNOP	Margarine (16.6%)	Processed meat (15.2%)	Fresh meat (11.7%)	43.5

II.3.4 Discussion

This study assessed the long-term dietary intake of eight phthalates and their most important food sources for Belgian adults. The highest intakes were found for DEHP, followed by DiBP and DnBP. The most important food group contributing to the DEHP intake was bread. In the worst case scenario, the highest DEHP concentration measured in all bread samples was used, i.e. 2,264 µg/kg. This highly contaminated bread was bought in a bakery in a city in the Northern part of Belgium. The fact that bread is a frequently consumed food in Belgium often bought by families in the same habitual bakery, creates a situation in which one systematically buys and consumes highly contaminated bread bought at the same bakery. Therefore, the contamination pathways of bread need to be investigated in detail in order to take actions to decrease the levels of phthalates (in particular DEHP) in bread (see also Chapter II.2). Moreover, it is clear that strategies to decrease the contamination of bread with phthalates will have a considerable effect on their intake. Besides bread, also other "healthy" food groups like fruits, vegetables and pulses were found to be important contributors to dietary phthalate exposure in Belgian adults. This may indicate that people with a healthy lifestyle are even more exposed to phthalates through their diet than other people. The same conclusion has been made in the studies of Dickson-Spillmann et al. (2009) and Sathyanarayana et al. (2013).

II.3.4.1 Comparison with other dietary intake assessments for phthalates

In this section, the results of the dietary intake assessment of phthalates for the Belgian population are compared to those reported for other populations. An overview of this comparison can be found in Table 27. However, this comparison needs to be interpreted with caution, due to differences between the studies in the analytical methods used to analyse phthalate concentrations, in the methodology used to collect food consumption data, and in the way the exposure results are expressed (mean versus median; per person versus per kg body weight). The assessed phthalate intakes of other studies are compared with the results of the medium bound probabilistic scenario, taking into consideration the effect of preparation, since this is most comparable to the approach used in the other studies.

Already in 1996, the UK Ministry of Agriculture, Fisheries and Food published dietary intakes of phthalates (BBP, DnBP, DEHP, DiBP) for the British adults, obtained via a total diet study (MAFF UK, 1996). It is however important to mention that they only considered food items from animal origin. Nevertheless, the intakes of BBP and DnBP were considerably higher in the English study compared to our study in adults in which both plant- and animal-based foods were considered. For DEHP, the British intakes were a bit higher and those for DiBP were in the same line as the Belgian adult intake levels (MAFF UK, 1996). In 2011, more recent data were published describing the dietary intakes of six phthalates (BBP, DnBP, DEHP, DEP, DiBP and DCHP) for the population of the UK based on food samples collected in 2007 (COT, 2011). Comparison of these more recent results with the Belgian intake results indicated that the assessed intakes for those six phthalates were comparable, being sometimes a bit higher in the UK compared to Belgium. Concerning the food sources of phthalates, some differences were found between the UK and Belgium. In the UK, bread was an important contributor for BBP, DnBP and DiBP but not for DEHP as for the Belgian population. Important sources of DEHP in the UK were fish, meat, poultry and dairy products (COT, 2011).

In 2000, dietary intake data of three phthalates (BBP, DnBP and DEHP) were published for the Danish adult population, based on a total diet study (Petersen and Breindahl, 2000). Similar to the English data, the Danish assessments for BBP and DnBP intake were higher compared to the Belgian results for adults. For DEHP, intakes from the Danish study were only slightly higher than the Belgian intakes.

In 2007, exposure results were published describing the DnPB, DEHP and DiBP intake of German adults (14 to 60 years old) based on a duplicate diet study (Fromme et al., 2007b). For all three phthalates, the intakes assessed for German adults were higher compared to the Belgian data for adults. For DnBP, median intake and 95th percentile were equal to respectively 0.3 and 1.4 µg/kg bw/day for the German adults compared to respectively 0.08 and 0.2 µg/kg bw/day for the Belgian adults. For DEHP, median intake and P95 was equal to respectively 2.4 and 4.0 µg/kg bw/day for the German adults compared to respectively 1.6 and 3.9 µg/kg bw/day for the Belgian adults. For DiBP, median intake and 95th percentile was equal to respectively 0.6 and 2.1 µg/kg bw/day for the German adults compared to respectively 0.2 and 0.5 µg/kg bw/day for the Belgian adults.

Dickson-Spillmann et al. (2009) published data on dietary intakes of BBP, DnBP, DEHP and DEP for Swiss adults. For all phthalates, median intakes for Swiss adults were higher than those assessed for Belgian adults, with exception of BBP. The median BBP intake for the Swiss versus the Belgian adults

was 0.03 versus 0.06 µg/kg bw/day, both far below the TDI for BBP, being 500 µg/kg bw/day (Dickson-Spillmann et al., 2009).

In general, it can be concluded that the majority of the dietary intake assessments for phthalates obtained in this Belgian study with the medium bound probabilistic scenario, taking into consideration the effect of preparation were lower or similar to the intakes reported in international literature. A possible reason for the lower intakes found in this study is the evolution in the intakes, due to replacement of several phthalates by alternatives. This was clearly shown by a recent German study investigating time trends in internal exposure to phthalates between 1998 and 2008, based on 24h-urine samples (Göen et al., 2011). They observed a decrease in exposure to DnBP (7-8 fold), DEHP (2-3 fold) and BBP (2-3 fold). However, an increase of DiNP exposure was found (4 fold) and the exposure to DiBP was found to be stable. These results indicate that there may be a shift in exposure from restricted phthalates to their substitutes.

Table 27: Overview of dietary phthalate intakes (in µg/kg bw/day) reported for adults in several studies.

Study	Population	Food type	P50	P95	P97.5	P99
DEP						
Dickson-Spillmann et al., 2009	Switzerland	Retail	0.02	-	-	-
COT, 2011	United Kingdom	Total diet	-	-	0.15-0.30	-
This study	Belgium	Retail	0.04	0.07	-	0.11
DiBP						
MAFF UK, 1996[2]	United Kingdom	Total diet (animal origin)	0.11[1]	-	0.26	-
Fromme et al., 2007b	Germany	Duplicate diet	0.6	2.1	-	-
COT, 2011	United Kingdom	Total diet	-	-	0.6-0.9	-
This study	Belgium	Retail	0.14	0.28	-	0.37
DnBP						
MAFF UK, 1996[2]	United Kingdom	Total diet (animal origin)	0.19[1]	-	0.44	-
Petersen and Breindahl, 2000[2]	Denmark	Total diet	1.9-4.1[1]	-	-	-
Fromme et al., 2007b	Germany	Duplicate diet	0.3	1.4	-	-
Dickson-Spillmann et al., 2009	Switzerland	Retail	0.39	1.17	-	3.83
COT, 2011	United Kingdom	Total diet	-	-	0.2-0.3	-
This study	Belgium	Retail	0.08	0.16	-	0.20
BBP						
MAFF UK, 1996[2]	United Kingdom	Total diet (animal origin)	0.11[1]	-	0.29	-
Petersen and Breindahl, 2000[2]	Denmark	Total diet	0.29-0.43[1]	-	-	-
Dickson-Spillmann et al., 2009	Switzerland	Retail	0.14	-	-	-
COT, 2011	United Kingdom	Total diet	-	-	0.04-0.50	-
This study	Belgium	Retail	0.05	0.12	-	0.16
DEHP						
MAFF UK, 1996[2]	United Kingdom	Total diet (animal origin)	2.14	-	4.29	-
Petersen and Breindahl, 2000[2]	Denmark	Total diet	2.70-4.29[1]	-	-	-
Fromme et al., 2007b	Germany	Duplicate diet	2.4	4.0	-	-
Dickson-Spillmann et al., 2009	Switzerland	Retail	1.90	-	-	-
COT, 2011	United Kingdom	Total diet	-	-	3.4-4.0	-
This study	Belgium	Retail	1.49	2.86	-	3.69
DCHP						
COT, 2011	United Kingdom	Total diet	-	-	0.03-0.20	-
This study	Belgium	Retail	0.02	0.04	-	0.06

-: not available; [1] Average instead of median intake values; [2] Intake values converted from *µg/person/day* to *µg/kg bw/day* using an estimated body weight of 70 kg (EFSA, 2012).

II.3.4.2 Limitations of the study

This study is the first one describing dietary phthalate intakes for a representative sample of Belgian adults, considering 12 different scenarios per phthalate and based on concentrations measured in more than 550 food samples. However, some limitations have to be discussed. First of all, although the consumption data used in this study are the most recent representative data for Belgian adults, they were collected eight years ago. Very likely consumption patterns have changed over the last ten years due to the presence of new food products on the market. For this study it was assumed that since 2004 the food consumption pattern of Belgian adults did not change substantially for the food groups contributing most to the phthalate exposure, i.e. bread, fruits and meat (Table 26). Moreover, the phthalate concentrations were measured in 572 food products that were bought on the market very recently (between 2009 and 2011), which is in line with the advice of Franco et al. (2007) stating that high quality monitoring data are needed for exposure estimates of phthalates.

Secondly, food consumption surveys aim at obtaining a detailed view of the dietary pattern of the study population, in this case adults. Nevertheless, food consumption data collected in such surveys can be under- or overreported by the study participants. Such bias is hard to overcome and will have an effect on the results of the intake assessment on individual level, but overall it can be assumed that such data give a good picture at population level.

Thirdly, for this project, concentrations of eight phthalates were analysed in 572 different food samples, which was laborious work, due to the complex methodology of sample handling and analysis, constantly avoiding external contamination, since these phthalates are ubiquitous. Nevertheless, this number was limited in comparison with the total number of food items present on the Belgian market that may contain phthalates, especially when considering the different brands and packaging materials that exist. For some particular food items (e.g. dried fruits, turkey), no concentration data were available. In those cases, the most logical and realistic alternative of the 572 analysed foods was used. For examples, phthalate concentration data of grapes were also used for raisins and data of chicken for turkey. This was to the authors' opinion the best possible way and is certainly better than assuming a zero concentration for those foods.

Next, the concentrations of DiNP and DiDP in foods were not considered in this study, since the laboratory did not have a validated method to assess these phthalates in food matrices by the time the analyses for this study were performed. However, DiNP and DiDP are more and more used to replace DEHP. This will result in lower intakes of DEHP in the future. Taking into account that DiNP and DiDP have received a tolerable daily intake value by EFSA (2005d; 2005e), it is desirable that future researchers will analyse these compounds in food products and assess and evaluate the dietary intake.

II.3.5 Conclusions

The dietary intake assessment of eight phthalates for the Belgian population indicated that DEHP had the highest long-term intakes, followed by DiBP. Risk characterisation for DEHP, BBP, DnBP and DEP showed that the assessed dietary intakes were far below the TDIs. Bread was an important source of DEHP exposure (and also of other phthalates). The contamination pathways for bread need therefore to be investigated in more detail (see also Chapter II.2).

II.4 Milk 1 – Analysis of phthalates in Belgian cow's milk at farm level

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S. and Sioen, I. (2012). ***Phthalates in Belgian cow's milk and the role of feed and other contamination pathways at farm level.*** *Food and Chemical Toxicology, 50, 2945-2953.*

Abstract

This study investigated the occurrence of dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP) in raw cow's milk and feed from Belgian farms in order to determine their most relevant contamination pathways in milk. Measurable levels of DMP, DEP, DnBP, DCHP and DnOP were found in various feed samples, although they were not observed in milk. A plausible explanation for this is that they are rapidly metabolised in cows. DEHP and in a smaller degree also DiBP and BBP levels in milk seemed to vary across seasons and farms. DiBP and BBP levels were lower in summer than in winter milk, which was in contrast with what was observed for DEHP. This is possibly due to another feed composition during summer and winter. Comparing BBP and DEHP concentrations in manually with those in mechanically obtained milk revealed that, besides environmental contamination via feed ingestion, contact materials used during the mechanical milking process is another important contamination pathway. Concentrations observed in this study confirm the decreasing trend of DEHP in European cow's milk owing to the substitution of DEHP by other plasticisers.

II.4.1 Introduction

Phthalates is the common generic name for diesters of ortho-phthalic acid (1,2-benzene dicarboxylic acid). These organic lipophilic compounds are produced in large volumes – the annual European production of phthalates is about one million tons – as constituents of a broad series of materials. According to their use, a distinction can be made between short (one till six carbon atoms in the alkyl chain) and long (more than six carbon atoms in the alkyl chain) alkyl chain phthalates (ECPI, 2010). Short alkyl chain phthalates such as dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP) and benzylbutyl phthalate (BBP) are typically used as solvent and can be present in adhesives, printing inks, personal care products and pharmaceuticals. Long alkyl chain phthalates (e.g. di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP)) are mainly used as plasticiser to soften polyvinyl chloride (Cao, 2010; CDC, 2009; Schettler, 2006; Stanley et al., 2003).

Due to their widespread use, numerous studies have investigated possible harmful effects of phthalates to the environment (Bradlee and Thomas, 2003; Parkerton and Staples, 2003) and to human health (Hauser and Calafat, 2005; Kamrin, 2009; Meeker et al., 2009; Shea, 2003). In Europe, risk assessment reports have been accomplished for DnBP, DEHP, BBP, DiDP and DiNP by the European Chemicals Bureau (ECB, 2003a; 2003b; 2004; 2007; 2008) and tolerable daily intakes (TDIs) have been specified for these phthalates by the European Food Safety Authority, namely 0.01 mg/kg bw for DnBP, 0.05 mg/kg bw for DEHP, 0.5 mg/kg bw for BBP, and 0.15 mg/kg bw for DiDP and DiNP (EFSA, 2005a; 2005b; 2005c; 2005d; 2005e). All this has led to a shift in the European market concerning the use of certain phthalates. For example, DEHP, of which it is known that it can disrupt the human endocrine system (Latini et al., 2004), has been more and more substituted by phthalates

or other plasticisers that have higher or no TDI values such as DiNP, DiDP and di(2-propylheptyl) phthalate (DPHP) (ECPI, 2010).

Human exposure to phthalates can occur via ingestion, inhalation, medical intravenous interventions or via dermal contact (Schettler, 2006). Numerous studies have indicated that for phthalates, and especially for DEHP, the intake of contaminated food is the most important exposure pathway for the general population (Clark et al., 2003a; Fromme et al., 2007b; Schettler, 2006; Shea, 2003; Wittassek et al., 2011; Wormuth et al., 2006). Phthalates are not only present in food because of an environmental transfer from soil, water or air, but also as a result of migration from phthalate containing contact materials used during cultivation, transport, production, storage or even during cooking at home (Blüthgen, 2003; Bradley et al., 2007; Cao, 2010; Du et al., 2009; Tsumura et al., 2001b). Milk and dairy products seem to make a major contribution to the daily dietary exposure to phthalates. For instance, Clark et al. (2003a) determined that milk and dairy products contribute for 17.2 and 27.6% to the total dietary DEHP exposure of Canadian adults and toddlers, respectively.

For the last two decades, various researchers have reported phthalate levels in milk and dairy products (Casajuana and Lacorte, 2004; Castle et al., 1990; Cousins and Mackay, 2001; Feng et al., 2005; Kim et al., 2009; Page and Lacroix, 1992; Petersen, 1991; Sharman et al., 1994; Sorensen, 2006). Six of them have considered the occurrence of phthalates in cow's milk at farm level (Castle et al., 1990; Cousins and Mackay, 2001; Feng et al., 2005; Kim et al., 2009; Sharman et al., 1994; Sorensen, 2006). DEHP was analysed in all six studies; other phthalate compounds (DMP, DEP, DnBP, BBP, di-*n*-octyl phthalate (DnOP), DiNP and/or DiDP) were only investigated in the three most recent ones. Seasonal variation of phthalate levels in raw cow's milk has not been studied yet. Concerning possible contamination pathways at farm level, cow's milk can be contaminated with phthalates during the mechanical milking process as a result of migration from the milking equipment. This has already been verified for DEHP by several researchers (Blüthgen, 2003; Castle et al., 1990; Feng et al., 2005; Ruuska et al., 1987; Wildbrett, 1973). Ingestion of polluted feed can also be a reasonable contamination pathway. However, studies concerning phthalate concentrations in feed are hard to find, which has already been mentioned by Jarosova et al. (2010), who revealed the presence of DEHP and DnBP in raw materials, premixes and feed additives.

In this study, the occurrence of phthalates in raw cow's milk and feed is investigated with the intention of describing the most relevant contamination pathways for phthalates in Belgian milk at farm level. This survey makes a contribution to the current knowledge about phthalate contamination of cow's milk in several ways. Firstly, besides well-investigated phthalates such as DEHP and to a certain extent also DMP, DEP, DnBP, BBP and DnOP, this study also examined phthalate compounds such as DiBP and dicyclohexyl phthalate (DCHP), which have not been analysed in raw cow's milk before. Secondly, phthalate concentrations in manually obtained milk were compared with concentrations in mechanically obtained milk in order to see if contamination via the milking equipment still takes place, despite the fact that there was a shift in the European market concerning the use of certain phthalates (e.g. DEHP). Thirdly, to meet the lack of data concerning phthalate levels in feed, this investigation also considers the occurrence of all eight mentioned phthalates in diverse feed samples. Fourthly, possible seasonal variation of phthalate levels in raw cow's milk is explored by analysing milk samples that were collected in summer as well as in winter. Finally, in order to gain more insight into a possible seasonal influence, phthalate intake by cows

through feed, soil and groundwater is calculated for both summer and winter. This survey is part of a larger study that investigated phthalate contamination in a whole Belgian milk chain (i.e. at farm, industry and retail level).

II.4.2 Material and methods

II.4.2.1 Sample collection

In Belgium – like in most European countries – it is common practice for farmers to milk their cows mechanically. The milking process can occur in the stable of the farm or in a milking parlour and usually starts with the inspection and cleaning of the cows' udders. Once the milking clusters are attached to the cows' teats, milk is extracted using a vacuum and is collected in a large cooling tank. Every 2 or 3 days, the cooling tank of the farm is emptied by a milk collecting company that delivers the milk to the dairy factories. A flow chart of all this is given in Figure 7.

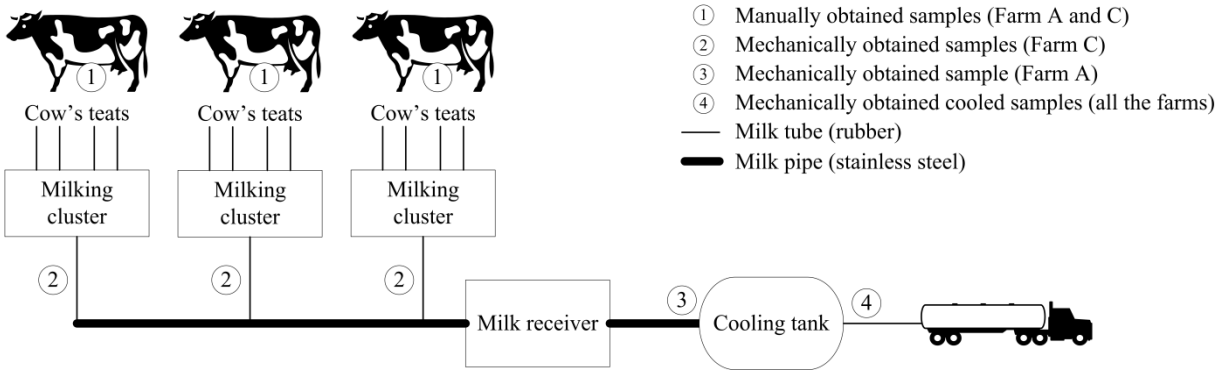

Figure 7: Flow chart of the milking process and the sampling places considered in this study.

Five farms (further referred to as "A", "B", "C", "D" and "E") participated in this survey. All farms were located in "The Kempen", a region in the northeast of Belgium, and were delivering to the same milk collecting company. The number of cows, all Holstein-Friesians, ranged between 40 and 80 per farm. Cows were on average 5 years old and produced milk with an average fat content of 4.5%. Cows' milking occurred twice a day (once in the morning and once in the evening) with the exception of farm D where cows were milked automatically once they had entered the milking parlour. At two farms (B and E), the milking process took place in the stables of the farms. During summer (from half of April until half of October), cows were grazing outside in pastures between the two milking processes (at farm D: 2 h a day). At night and during winter, cows were inside the stables standing or laying on grid floors. At all farms, groundwater was pumped up and was used as drinking water for the cows. The brand of the milking system and the cooling tank was, except for the milking system of farm B, the same for all farms. The milking system consisted of rubber tubes, stainless steel pipes, milking clusters of rubber and stainless steel and a milk receiver made from hard plastic, stainless steel and rubber. The cooling tank was made from stainless steel and contained a few rubber sealants, e.g. on the manhole cover and on the tap of the cooling tank. The milking system was cleaned after each milking process (at farm D: three times a day) and the cooling tank after every time the tank was emptied. The cleaning process occurred automatically by means of a cleaning unit with hot water and a basic (six out of seven times) or an acidic (one out of seven times) solution.

To investigate seasonal variation, raw milk samples from the cooling tank (point 4 in Figure 7) of the five farms were collected at four different moments: twice during summer 2010 and twice during winter 2010-2011. At farm A and C, additional milk samples were taken at different sampling places during the milking process, namely samples of milk milked by hand, by machine and from the cooling tank, in order to explore if migration of phthalates from contact materials used during the mechanical milking process takes place. At these two farms, samples of feed (silage, pasture and concentrate), groundwater and soil were taken as well, to verify if milk can be contaminated with phthalates via the environment.

At farm A, individual milk samples (point 1 in Figure 7) from ten cows were obtained manually in winter 2010-2011 (another day than the two sampling days described in the previous paragraph). After the collection of the manually obtained samples, a mechanically obtained milk sample from these ten cows (pooled sample) and a milk sample from the cooling tank were collected at the same day as well. The mechanically obtained pooled sample was tapped just before the milk entered the cooling tank (point 3 in Figure 7). At farm C, manually and mechanically obtained raw milk samples were collected from four cows in winter 2009-2010 (point 1 and 2 in Figure 7). Manually obtained samples were taken before the mechanical milking process started; mechanically obtained samples were collected from the plastic vessels of the milk meters that were connected during milking. Just like at farm A, a raw milk sample from the cooling tank was taken during this sampling moment as well. Feed samples at farm A consisted of silage (pooled sample of grass silage, maize silage, beet pulp, soya meal and triticale silage) and pasture while feed at farm C was composed of silage (pooled sample of grass silage, maize silage and soya bean meal), pasture and concentrate. Concentrate and silage samples were collected in winter 2010-2011 and samples of pasture, soil and groundwater were gathered in summer 2011. Silage, concentrate and groundwater samples were taken in the stable of the farms, more exactly from the concrete floor, the crib and from a tap, respectively. Since cows also ingest soil particles when they are grazing, soil samples were taken from the first 30 cm of the pastures.

At every sampling moment, hands were cleaned with water and no gloves were used. For each sample, three glass bottles (Duran) with polypropylene screw caps were filled with at least 100 ml of raw milk, 100 g of feed/soil or 500 ml of groundwater. The bottles were transported to the laboratory in a cool box and stored at –18 °C prior to analysis. In order to avoid sample contamination, the screw caps of the bottles were covered on the inside with aluminium foil.

II.4.2.2 Analytical procedure

The sample preparation and analysis of DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP in raw cow's milk, feed, soil and groundwater were performed according to the method described by Fierens et al. (2012c). Briefly, the sample preparation method of high-fat foods was used to prepare raw cow's milk samples. Of every milk sample, 40 g was prepared to obtain at least 0.5 g milk fat after extraction. Feed and soil samples were prepared in the same way as low-fat foodstuffs. The amount of silage and pasture samples was 5 g. For the preparation of soil and concentrate samples, 10 g was used. Finally, 500 ml of every groundwater sample was prepared following the preparation method that was developed for aqueous-based beverages. All samples, with the exception of groundwater samples, were purified via gel permeation chromatography (GPC). Instrumental analysis was performed by gas chromatography-low resolution-mass spectrometry with electron impact

ionisation (GC-EI-MS). Detection of the different phthalate compounds took place in selected ion monitoring (SIM) mode. For all phthalates except DMP, the dominant product ion *m/z* 149 was used as target ion; for DMP, *m/z* 163 was used. As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compound in a sample had to be within 20% of that observed in a standard solution. Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (12 samples at the most) to reduce the risk of contamination.

The analytical procedure was proven to be well reproducible (Fierens et al., 2012c). Recoveries varied between 88% and 104% and the relative standard deviation (RSD) for each phthalate compound in the different matrices was lower than 13%. The measurement uncertainty, calculated as the sum of the average recovery bias and two times the RSD value, was lower than 30% for each phthalate compound.

Because phthalates are ubiquitous, limits of quantification (LOQs) were strongly dependent on the feasible blank concentrations. Therefore, LOQ calculations were based on the phthalate concentrations detected in the procedural blanks. LOQs were separately calculated for each phthalate compound and for each kind of matrix and equalled the sum of the average blank concentration and six times the standard deviation of replicate procedural blank measurements under reproducibility conditions (each replicate determination was obtained from an independent extraction). LOQs varied between 5 and 60 µg/kg fat for raw milk samples (based on 28 procedural blanks), between 0.1 and 1.4 µg/kg fresh weight for silage and pasture samples (based on six procedural blanks), between 0.05 and 0.50 µg/kg fresh weight for soil and concentrate samples (based on four procedural blanks) and finally, between 0.01 and 0.35 µg/kg fresh weight for groundwater samples (based on four procedural blanks). At every sampling point, three samples were collected in separate glass bottles and every bottle was analysed once. So, in total, three concentrations were obtained for each phthalate compound at each sampling point, of which the average was calculated. When it was obvious that contamination had occurred – i.e. when the difference between two phthalate concentrations of the triplicates amounted more than the LOQ value – the highest phthalate concentration was not taken into account.

Because the fat content of milk can vary between farms, cows and seasons, phthalate concentrations in raw cow's milk were reported in this survey on fat basis (in *µg/kg fat*). In this way, phthalate levels in milk samples from different farms, cows and obtained in different seasons could be better compared regardless differences in fat content. All other samples were reported on whole weight basis (in *µg/kg fresh weight*).

II.4.2.3 Calculation of phthalate exposure through ingestion of feed, soil and groundwater

Exposure through the ingestion of feed, soil and groundwater (E_{diet}; in *µg/(cow day)*) during winter 2010-2011 and summer 2011 was calculated for a cow at farm A and C for every phthalate compound using Equation 4:

Equation 4

$$E_{diet} = \left[\sum_{i=1}^{n}(q_{feed_i} \times C_{feed_i})\right] + (q_{soil} \times C_{soil}) + (q_{water} \times C_{water})$$

where *q* is the amount of feed *i* (silage, pasture and concentrate), soil or groundwater consumed daily (in *kg fresh weight/(cow day)*) and *C* is the phthalate concentration in feed *i*, soil or groundwater (in *µg/kg fresh weight*). Intake amounts of feed, soil and groundwater for a cow at farm A and C during winter 2010-2011 and summer 2011 are shown in Table 28. The amounts were received from the two corresponding farms or, if unknown, were estimated by means of data reported in other surveys (ILVO, 2011; Mayland et al., 1975; Van Raamsdonk et al., 2007). When a phthalate compound was not detected in feed, soil or groundwater, the concentration of that compound was set at zero in the calculations. Calculated exposure rates resulting from phthalate concentrations that were below the LOQ value, were divided by two to calculate total exposure rates.

Table 28: Intake amounts of feed, soil and groundwater for a cow at farm A and farm C during winter 2010-2011 and summer 2011 (in kg fresh weight/(cow.day)).

Intake amount of	Winter 2010-2011	Summer 2011	Reference
Farm A			
Silage	53	34	Farm A
Pasture	0	49	Van Raamsdonk et al., 2007
Soil	0	0.8	Mayland et al., 1975
Groundwater	65	65	ILVO, 2011
Farm C			
Silage	32	17	Farm C
Pasture	0	49	Van Raamsdonk et al., 2007
Concentrate	6	6	Farm C
Soil	0	0.8	Mayland et al., 1975
Groundwater	65	65	ILVO, 2011

II.4.3 Results

II.4.3.1 Phthalate concentrations in summer and winter milk

Phthalate concentrations found in mechanically obtained summer and winter milk from the cooling tanks of five farms are given in Table 29. In all samples, DMP, DEP, DCHP and DnOP were not detected. DnBP could only be quantified in one summer milk sample. DiBP was only measurable in the winter milk of four farms, ranging from 17.2 to 51.5 µg/kg fat. Concentrations of BBP above the LOQ were found in one summer milk sample (15.5 µg/kg fat) and in four winter milk samples (15.0; 16.1; 17.3 and 20.5 µg/kg fat). DEHP could be quantified in all milk samples, except in one summer milk sample of farm C. DEHP levels varied during summer from not detected to 788 µg/kg fat (average concentration of 400 ± 255 µg/kg fat) and between 201 and 500 µg/kg fat during winter (average concentration of 298 ± 85 µg/kg fat).

Table 29: Concentrations of eight phthalates (average of day 1 – average of day 2) in mechanically obtained summer and winter milk from the cooling tank of five farms (in µg/kg fat).

Origin	N [a]	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Summer 2010									
Farm A	2	ND	ND	ND-<15	ND	ND	664-670	ND	ND
Farm B	2	ND	ND	ND-<15	ND-<15	ND-15.5	298-302	ND	ND
Farm C	2	ND	ND	<15	ND-<15	ND	ND-66	ND	ND
Farm D	2	ND	ND	ND-<15	ND-15.3	ND-<10	419-788	ND	ND
Farm E	2	ND	ND	ND	ND	ND-<10	360-436	ND	ND
Winter 2010-2011									
Farm A	2	ND	ND	ND-<15	ND-<15	<10	316-351	ND	ND
Farm B	2	ND	ND	24.2-28.3	<15	17.3-20.5	249-304	ND	ND
Farm C	2	ND	ND	22.1-26.3	ND-<15	<10-15.0	257-321	ND	ND
Farm D	2	ND	ND	30.9-51.5	<15	<10-16.1	201-500	ND	ND
Farm E	2	ND	ND	17.2-18.4	ND	<10	228-257	ND	ND

ND: not detected; <value: detected, but lower than LOQ value; [a] Number of sampling days; at every sampling day, three glass bottles of raw cow's milk from the cooling tank were collected and every bottle was analysed once; LOQs: 5 µg/kg fat (DMP), 10 µg/kg fat (BBP and DnOP), 15 µg/kg fat (DiBP, DnBP and DCHP), 20 µg/kg fat (DEP) and 60 µg/kg fat (DEHP).

II.4.3.2 Phthalate concentrations in manually and mechanically obtained winter milk

Table 30 shows phthalate levels in cow's milk, milked by hand and by machine and coming from the cooling tank of farm A and farm C. Just like in the summer and winter samples, DMP, DEP, DCHP and DnOP were not detected. The concentration of DiBP at farm A amounted 29.0 µg/kg fat in manually obtained milk (median concentration), 21.5 µg/kg fat in mechanically milked milk and 22.0 µg/kg fat in milk from the cooling tank. At farm C, DiBP was mostly not detected. DnBP could only be quantified in a few samples of manually obtained milk from farm A. BBP was measured in levels above the LOQ in mechanically obtained milk from farm A and farm C, namely 18.0 and 14.3 µg/kg fat, respectively. At both farms, BBP was not detected in milk from the cooling tank and median levels in milk milked by hand were below the LOQ. Median concentrations of DEHP in manually obtained milk from both farm A and C were below the LOQ. DEHP in milk milked by machine from farm A was even not detected. On the other hand, DEHP could be measured in mechanically obtained milk from farm C (median concentration of 124 µg/kg fat) and in milk from the cooling tanks of both farms (338 µg/kg fat at farm A and 119 µg/kg fat at farm C).

Table 30: Concentrations of eight phthalates (min. – max. (median)) in cow's milk milked by hand, by machine and from the cooling tank of farm A and farm C (in µg/kg fat).

Milk type	N [a]	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Farm A (winter 2010-2011)									
Milked by hand	10	ND	ND	ND-123 (29.0)	ND-25.2 (<15)	ND-37.2 (<10)	ND-91 (<60)	ND	ND
Milked by machine	1 [b]	ND	ND	21.5	<15	18.0	ND	ND	ND
Cooling tank	1	ND	ND	22.0	ND	ND	338	ND	ND
Farm C (winter 2009-2010)									
Milked by hand	4	ND	ND	ND-<15 (ND)	ND-<15 (ND)	<10-16.3 (<10)	ND-<60 (<60)	ND	ND
Milked by machine	4	ND	ND	ND-15.1 (ND)	ND	ND-44.6 (14.3)	76-162 (124)	ND	ND
Cooling tank	1	ND	ND	ND	ND	ND	119	ND	ND

ND: not detected; <value: detected, but lower than LOQ value; [a] Number of samples; at every sampling point, three glass bottles of raw cow's milk were collected and every bottle was analysed once; [b] A pooled sample of ten cows was taken before the milk entered the cooling tank; LOQs: 5 µg/kg fat (DMP), 10 µg/kg fat (BBP and DnOP), 15 µg/kg fat (DiBP, DnBP and DCHP), 20 µg/kg fat (DEP) and 60 µg/kg fat (DEHP).

II.4.3.3 Phthalate concentrations in feed, soil and groundwater

Analytical results of feed, soil and groundwater samples from farm A and C that were taken in winter 2010-2011 or summer 2011, are given in Table 31. In groundwater from both farm A and C, none of the investigated phthalates were detected. On the other hand, in silage and concentrate, all eight phthalates (with the exception of DnOP in silage from farm A) were detected at concentrations higher than the corresponding LOQ values. In pasture from farm A, only DEHP could be measured above the LOQ (21.9 µg/kg fresh weight), whereas in pasture from farm C, concentrations of DMP, DiBP, DnBP, DCHP and DnOP were also found. Furthermore, DMP, DnBP, BBP and DEHP were observed in soil samples from both farms, while DCHP and DnOP were only found in levels above the LOQ in soil from farm A.

Table 31: Phthalate concentrations in feed, soil and groundwater collected at farm A and C in winter 2010-2011 or summer 2011 (in µg/kg fresh weight).

Sample type	N [a]	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Farm A									
Silage	1	7.76	4.14	14.9	7.62	17.1	32.9	0.82	<0.20
Pasture	1	ND	ND	<1.40	<1.15	ND	21.9	ND	<0.15
Soil	1	0.28	ND	<0.70	1.58	0.45	12.2	0.43	0.12
Groundwater	1	ND	ND	ND	ND	ND	ND	ND	ND
Farm C									
Silage	1	0.24	3.92	3.41	18.6	21.5	15.2	1.43	1.48
Pasture	1	0.15	<0.45	2.37	3.23	<0.35	13.9	0.36	0.19
Concentrate	1	0.24	0.65	3.23	0.80	16.6	17.3	0.27	0.22
Soil	1	0.06	ND	<0.70	2.67	0.39	4.49	<0.15	ND
Groundwater	1	ND	ND	ND	ND	ND	ND	ND	ND

ND: not detected; <value: detected, but lower than LOQ value; [a] Number of samples; at every sampling point, three glass bottles were collected and every bottle was analysed once; LOQs in groundwater: 0.01 µg/kg (DMP, BBP, DCHP and DnOP), 0.05 µg/kg (DEHP), 0.1 µg/kg (DEP and DnBP) and 0.35 µg/kg (DiBP).

II.4.3.4 Phthalate exposure through ingestion of feed, soil and groundwater

Phthalate exposure through the ingestion of feed, soil and groundwater for a cow at farm A and C during winter 2010-2011 and summer 2011 is given in Table 32. Calculations were based on intake amounts of feed, soil and groundwater at both farms (Table 28) and on phthalate concentrations measured in the corresponding samples (Table 31). Oral exposure to DMP, DEP, DiBP, BBP and DEHP was higher at farm A than at farm C, whereas cows from farm C were exposed to DnBP and DnOP at a higher degree than cows from farm A. Exposure to DCHP was more or less the same at both farms. Table 32 also shows that exposure during summer and winter can differ. For farm A as well as for farm C, exposure to DEP, DnBP and BBP was higher during winter than during summer, while the opposite was observed for exposure to DEHP. Similar exposure rates during summer and winter were found for DCHP and DnOP at farm A and farm C. At farm A, exposure to DMP and DiBP was higher in winter than in summer. On the other hand, at farm C, exposure to DMP during winter was nearly the same as during summer and exposure to DiBP was lower in winter than in summer. The contribution of soil and groundwater to phthalate exposure was negligible compared to the contribution of silage, pasture and concentrate at both farms.

Table 32: A cow's phthalate exposure through the ingestion of feed, soil and groundwater during winter 2010-2011 and summer 2011 at farm A and farm C (in µg/(cow day)). When a phthalate compound was not detected in feed, soil or groundwater, the concentration of that compound was set at zero in the calculations. Calculated exposure rates resulting from phthalate concentrations that were below the LOQ value (indicated with "<"), were divided by two to calculate total exposure rates.

Exposure through	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Farm A (winter 2010-2011)								
Silage	412	219	791	404	905	1,745	43.6	<10.6
Groundwater	0	0	0	0	0	0	0	0
TOTAL	412	219	791	404	905	1,745	43.6	5.3
Farm A (summer 2011)								
Silage	264	141	508	259	580	1,119	28.0	<6.8
Pasture	0	0	<68	<56	0	1,063	0	<7.2
Soil	0.2	0	<0.6	1.2	0.3	9.2	0.3	0.1
Groundwater	0	0	0	0	0	0	0	0
TOTAL	264	141	542	288	580	2,191	28.3	7.1
Farm C (winter 2010-2011)								
Silage	7.5	123	107	582	675	478	45.1	46.5
Concentrate	1.4	3.9	19.4	4.8	99	104	1.6	1.3
Groundwater	0	0	0	0	0	0.	0	0
TOTAL	8.9	127	126	587	774	582	46.7	47.8
Farm C (summer 2011)								
Silage	4.0	65.7	57.1	310	360	255	24.0	24.8
Pasture	7.5	10.9	115	157	8.5	673	17.6	9.0
Concentrate	1.4	3.9	19.4	4.8	99	104	1.6	1.3
Soil	0.1	0	<0.6	2.0	0.3	3.4	<0.2	0
Groundwater	0	0	0	0	0	0	0	0
TOTAL	13.0	80.5	192	474	468	1,035	43.3	35.1

II.4.4 Discussion

In this study, the occurrence and concentration levels of eight phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) were determined in raw cow's milk, feed, soil and groundwater, coming from different Belgian farms. Possible contamination sources to explain the occurrence of these phthalates in raw cow's milk are formulated in the next sections.

II.4.4.1 Occurrence of DMP, DEP, DnBP, DCHP and DnOP in raw cow's milk

Although levels higher than the LOQ values of DMP, DEP, DnBP, DCHP and DnOP were found in various feed samples (Table 31), they were – with the exception of DnBP in a few samples from farm A – not observed in manually obtained milk (Table 30). A plausible explanation for these observations is that these phthalates are rapidly metabolised in cows. For instance, in humans and other mammalians, it is known that short alkyl chain phthalates such as DnBP are metabolised and excreted as monoesters, mostly in urine, within 48 h (Frederiksen et al., 2007). Furthermore, levels of DMP, DEP, DnBP, DCHP and DnOP in mechanically obtained milk and milk from the cooling tank

were, except for DnBP in one summer milk sample, also not above the LOQ (Table 29 and Table 30). This indicates that either the contact materials used at the participating farms do not contain these compounds or that the five mentioned phthalates do not migrate into the milk in concentrations that are detectable.

II.4.4.2 Occurrence of DiBP in raw cow's milk

The occurrence of DiBP in milk from the cooling tank of the five farms was different during summer (not detected or below the LOQ value) than during winter (measurable at four of the five farms) (Table 29). Furthermore, differences in DiBP concentrations between milk milked by hand and by machine were noticed at farm A (Table 30). Since quantifiable levels of DiBP were observed in manually obtained milk (median concentration of 29 µg/kg fat), it can be concluded that at this farm, DiBP contamination occurs via the environment, more exactly via the ingestion of contaminated silage (Table 32). As no DiBP could be quantified in the soil sample from farm A, the considered silage is almost certainly contaminated with DiBP due to migration from contact materials such as cling films, sails or sealants used during production, mixing or during storage at the farm (Cao, 2010; CDC, 2009). The results also show that, at farm A, manually milked milk contains more DiBP than mechanically milked milk or milk from the cooling tank (Table 30). It is possible that, besides contamination via feed intake, DiBP contamination at farm A also occurs through e.g. the use of a disinfectant during the inspection and cleaning of the cows' udders. Since the manually obtained samples were taken before the mechanically obtained ones, it is possible that the manually obtained samples still contained traces of e.g. this disinfectant, which were not present anymore in the mechanically obtained samples. Something that also has to be taken into account is the origin of the milk samples that were taken at farm A: manually obtained samples were deriving from ten cows separately, the mechanically obtained sample was a pooled sample of these ten cows and the sample from the cooling tank was a pooled sample from all the cows present at this farm. At farm C, no differences were noticed between milk milked by hand, by machine and milk from the cooling tank. In fact, in all samples, DiBP was not detected (based on median samples). Thus, DiBP contamination via the environment seems not to occur at farm C or at least in a minor degree than observed at farm A. This can also be concluded from the exposure rates that were calculated for a cow at farm A and C (Table 32): a cow at farm A is exposed to DiBP at a higher degree than a cow at farm C during winter, namely 791 compared to 127 µg/(cow day).

II.4.4.3 Occurrence of BBP in raw cow's milk

Raw cow's milk from the cooling tank was slightly higher contaminated with BBP during winter than during summer (Table 29). At farm A and C, the same trend was observed for BBP exposure via dietary intake (Table 32), which was mainly caused by the ingestion of silage. Possible contamination pathways for silage might be the use of BBP in contact materials such as cling films, sealants or tubes used for the production, mixing or storage at the farms. Silage can also be contaminated with BBP via the environment, for instance via uptake from the soil or via deposition (Cao, 2010; CDC, 2009; ECB, 2007; Williams, 2010). However, environmental contamination of silage can almost be ignored, since the levels of BBP that were determined in the other environmental samples (pasture, soil and groundwater) from farm A and C (Table 31) were rather low or even not quantifiable. At both farm A and C, mechanically obtained milk contained more BBP than manually obtained milk (Table 30; based on median concentrations of BBP). This may lead to the conclusion that at these farms, cow's milk is also contaminated with BBP during the mechanical milking process as a result of migration from

contact materials. Milk milked by machine contained surprisingly more BBP than milk from the cooling tank of both farm A and C (Table 30). This is unusual, but can be owing to the fact that the two types of samples have a different origin, i.e. mechanically obtained milk samples were taken from a few cows and milk samples from the cooling tank were pooled samples of all the cows that were present at the farms.

II.4.4.4 Occurrence of DEHP in raw cow's milk

In this survey, DEHP was by far the most and highest detected phthalate compound. In the following paragraphs, possible contamination pathways are described to explain the occurrence of DEHP in summer and winter milk as well as in manually and mechanically obtained milk.

Table 30 shows that DEHP could generally not be quantified in manually obtained winter milk. On contrary, measurable levels of DEHP were determined in winter (and summer) milk collected from the cooling tank (Table 29), which indicates that DEHP contamination in milk takes place during the mechanical milking process as a result of migration from contact materials (this issue is discussed more into detail in the next paragraph). Table 29 also illustrates that DEHP levels in winter milk were in general lower than in summer milk, namely 298 compared to 400 µg/kg fat on average. On the assumption that migration of DEHP from contact materials into cow's milk during winter and summer is equal, it can be concluded that this seasonal variation is the result of DEHP transfer via the environment (i.e. DEHP exposure through the ingestion of feed, soil or groundwater). The calculated exposure rates that are given in Table 32 confirm this conclusion: cows from farm A and C are less exposed to DEHP via ingestion during winter than during summer. At both farm A and C, the largest contributions to DEHP exposure through ingestion were on the account of silage and pasture (Table 32). As already mentioned in the previous sections, feed can be contaminated with DEHP as a result of migration from contact materials (Cao, 2010; CDC, 2009) or via environmental transfer (Blüthgen, 2003; Cousins and Mackay, 2003; Staples et al., 1997), which is exclusively the case for pasture.

At farm C, milk samples obtained by machine contained at least twice as much DEHP than manually obtained milk (Table 30). In addition, no increase in DEHP concentration was observed from mechanically obtained milk (point 2 in Figure 7) to milk from the cooling tank (point 4 in Figure 7). These results imply thus that at farm C, besides contamination through feed intake, milk is also contaminated with DEHP as a result of migration from plasticised contact materials such as milk tubes or sealants. This conclusion has already been reported by other researchers as well (Blüthgen, 2003; Castle et al., 1990; Feng et al., 2005; Ruuska et al., 1987; Wildbrett, 1973). Furthermore, Table 30 shows that, at farm A, DEHP levels only increased (with at least a factor six!) from mechanically obtained milk (point 3 in Figure 7) to milk from the cooling tank (point 4 in Figure 7). This leads to the conclusion that at farm A, DEHP contamination only occurs in the cooling tank. This was not expected, since the cooling tank from farm A (and from the other farms as well) only contained a few components (i.e. sealants) that could contain DEHP. Considering farm dependency, differences in DEHP concentrations were noticed between the mechanically obtained milk samples from farm A and C (not detected compared to 124 µg/kg fat, respectively) and between the cooled milk samples (338 compared to 119 µg/kg fat, respectively), although the brand of the milking system and the cooling tank was the same at both farms. Maybe, DEHP concentrations in mechanically obtained milk differed between farm A and C owing to the fact that the milking system at farm A is older than the system used at farm C (i.e. DEHP possibly did not migrate anymore from the milking system into the

milk at farm A). Another reason for this difference can be that, at farm C, milk was extra contaminated with DEHP via migration from the milk meters (with a.o. plastic vessels and rubber tubes) that were used during sampling. Something that can influence DEHP concentrations in both mechanically obtained milk samples and milk samples from the cooling tank, is the use of other cleaning agents at farm A and C. Anyway, the conclusion can be made that milk is contaminated with DEHP during the mechanical milking process, although contamination pathways seem to be farm dependent.

II.4.4.5 Comparison with other studies

In this section, phthalate concentrations obtained in this study are compared with results from other studies. Unfortunately, this was only possible for concentrations in cow's milk, soil and groundwater, as no studies could be found that report phthalate concentrations in silage, pasture or concentrate. When the fat content of the investigated milk samples was not mentioned in a study, the average European fat content of raw cow's milk, namely 4.04%, was used to calculate concentrations in *µg/kg fat* from whole weight based results (Eurostat, 2012). Furthermore, since the time of sampling (summer or winter) was not reported in other surveys, phthalate concentrations in summer and winter milk of this study were gathered.

Up till now, the occurrence of phthalates in manually obtained raw cow's milk has been considered in only two studies: a Norwegian (Castle et al., 1990) and a Canadian (Feng et al., 2005) one. Median DEHP levels in manually obtained Norwegian cow's milk were, just like in this study, below the LOQ. However, it should be noticed that the LOQ value from the Norwegian study was about two times higher than the LOQ value obtained in this study, namely 124 µg/kg fat compared to 60 µg/kg fat, so an adequate comparison could not be made. In the Canadian survey, similar concentrations of DMP, DEP, BBP and DnOP compared to this study were found. On contrary, much higher levels of DnBP and DEHP were determined, namely 180 and 451 µg/kg fat in the Canadian study compared to not detected or <15 and <60 µg/kg fat in this study, respectively. This contrast might be due to the fact that the regulation of phthalates in Canada and Europe differ (Wordsworth, 2007). Moreover, the Canadian study was conducted 5 years earlier than this study and during these 5 years, legislations concerning the use of phthalates such as DnBP and DEHP have been tightened, especially in Europe, e.g. their use in toys, childcare articles and cosmetic products (Official Journal of the European Union, 2005; SCCP, 2007).

Table 33 summarises several studies that have analysed DEHP in mechanically obtained raw cow's milk. A distinction is made between European (Castle et al., 1990; Cousins and Mackay, 2001; Sharman et al., 1994; Sorensen, 2006 and this survey) and non-European studies (Feng et al., 2005; Kim et al., 2009). First of all, the results in Table 33 clearly show that, in Europe, DEHP levels in raw cow's milk have decreased since the early nineties until now. This decline is most likely due to the fact that DEHP has been more and more substituted by other plasticisers during the last decade (ECPI, 2010). From 2001 till now, DEHP concentrations in European cow's milk seem to remain constant. In contrast, DEHP concentrations in milk from outside Europe are still very high, namely 1,411 µg/kg fat on average in milk from South Korea (Kim et al., 2009) and 5,357 µg/kg fat in Canadian milk (Feng et al., 2005). These concentrations are, respectively, about five and seven times higher than concentrations that have been found in recent European studies (Cousins and Mackay,

2001; Sorensen, 2006 and this survey). This discrepancy is probably due to differences in legislation regarding phthalates in food and food contact materials.

Table 33: Comparison of DEHP concentrations in raw cow's milk from cooling tanks obtained in several studies (in µg/kg fat).

Study	Country	N [a]	Average	Min.	Median	Max.
European studies						
Castle et al., 1990	Norway	6	1,237 [b]	743 [b]	1,300 [b]	1,980 [b]
Sharman et al., 1994	Norway	3	3,667 [c]	3,333 [c]	3,667 [c]	4,000 [c]
Cousins and Mackay, 2001	The Netherlands	29	-	-	670	-
Sorensen, 2006	Denmark	18	-	173 [b]	-	743 [b]
This study, 2010-2011	Belgium	20 [d]	351	ND	310	788
Non-European studies						
Feng et al., 2005	Canada	6 [e]	5,357 [c]	4,136 [c]	5,796 [c]	7,347 [c]
Kim et al., 2009	South Korea	30	1,411 [b]	ND	-	3,812 [b]

ND: not detected; [a] Number of samples; [b] Calculated from whole weight based results with an estimated fat percentage of 4.04 % (Eurostat, 2011); [c] Calculated from whole weight based results with reported fat percentages; [d] Results of summer and winter milk together; all samples were analysed in triplicate; [e] Results of individual mechanically obtained milk samples from six cows.

As already mentioned in Section II.4.1, only three studies (Feng et al., 2005; Kim et al., 2009; Sorensen, 2006) have also analysed other phthalate compounds than DEHP in mechanically obtained raw cow's milk. For instance, DMP was detected in South Korean milk with an average concentration of 24.8 µg/kg fat (Kim et al., 2009), while DMP in Canadian milk (Feng et al., 2005) and in milk from this study was not detected. On contrary, DEP was detected in the study of Feng et al. (2005) with an average concentration of 15.7 µg/kg fat, while DEP was not detected in this study and in the South Korean study (Kim et al., 2009). Both studies could not detect DnOP, which was also the case for this survey. Furthermore, DnBP was analysed in all three mentioned studies, but could only be quantified in the non-European studies, namely 144 µg/kg fat in the Canadian (Feng et al., 2005) and 743 µg/kg fat in the South Korean study (Kim et al., 2009). Since in this study the LOQ value for DnBP in cow's milk amounted 15.0 µg/kg fat, which was only achieved once (concentration of 15.3 µg/kg fat in one summer milk sample), the conclusion can be made that European milk is less contaminated with DnBP than non-European milk. In general, BBP could not be quantified in this study, which was also the case in the three other mentioned studies. None of the cited studies have investigated the occurrence of DiBP or DCHP in cow's milk, so no comparison could be made for these phthalate compounds.

A short literary study was performed in order to find out if concentrations of phthalates in soil and groundwater determined in this study are comparable with concentrations reported in other European studies. Gibson (2005), for instance, reported levels of DMP, DEP, DnBP, BBP, DEHP and DnOP determined in British agricultural soils. While DMP and BBP concentrations were similar to the concentrations obtained in this study, higher levels of DEP, DnBP, DEHP and DnOP were observed in the British study. Higher levels of DnBP and DEHP were also reported for European soils by Clark et al. (2003b). These two differences are probably owing to the fact that the use of phthalates has been limited in Europe for several years (Official Journal of the European Union, 2005; SCCP, 2007). Clark et al. (2003b) also described the occurrence of DMP, DnBP, BBP, DEP and DEHP in European

groundwater. Again, higher concentrations were observed, except for DMP, which could, just like in this study, not be detected.

II.4.4.6 Strengths and weaknesses

Up to now, only a few researchers have investigated the presence of phthalates, other than DEHP, in raw cow's milk. Moreover, phthalates like DiBP and DCHP have not been examined yet. Even more unexplored are the occurrence of phthalates in feed and the influence of seasonal variation on phthalate levels in cow's milk. To meet this lack of data, in this survey, the occurrence of eight phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) was investigated in raw cow's milk, sampled in summer and in winter as well as at different places during the milking process, and in feed deriving from different Belgian farms in order to explore their most relevant contamination pathways in raw cow's milk.

Because the presence of phthalates in the environment is ubiquitous and because phthalates have numerous user applications, it was very difficult to draw concrete conclusions regarding contamination pathways in raw cow's milk and feed. Moreover, it was hard to evaluate the obtained results in a critical way, since only a few studies could be found that have done comparable research. Therefore, it is important that the possible contamination pathways that are formulated in this study will be explored in future studies as well.

The fact that sample contamination with phthalates can occur in every step of the analytical procedure makes analysing phthalates very hard. In this study, the decision was made to analyse every sample in triplicate, i.e. three bottles were collected and every bottle was analysed once. When the difference between two phthalate concentrations of the triplicate samples amounted more than the LOQ value, the highest phthalate concentration was rejected. Furthermore, due to the numerous precautionary measures that have to be taken to avoid sample contamination and due to the several analytical steps, the analysis of phthalates is time-consuming, which limited the amount of samples that could be measured in this study. For instance, manually and mechanically obtained milk samples from individual cows were only examined at two of the five farms and only during winter. Furthermore, milk samples from the cooling tanks of the five farms were only taken from one year (mainly due to time constraints), which means that conclusions regarding seasonal variations are difficult to generalise.

II.4.4.7 Future research

Through this study, new data concerning phthalates in raw cow's milk and feed were provided and possible contamination pathways for phthalates in milk were discussed (e.g. DiBP contamination through feed). Nevertheless, further research is still desirable, especially in order to obtain more data concerning phthalates, other than DEHP, in cow's milk and feed and in order to explain their occurrence in cow's milk.

The influence of seasonal variation on phthalate levels in raw cow's milk has to be considered in other studies as well, since this study was the first to notice differences in phthalate concentrations and dietary exposure rates between summer and winter.

This study demonstrated that cows were exposed to DMP, DEP, DnBP, DCHP and DnOP via dietary intake. However, these five phthalates were not present in manually obtained cow's milk, probably

due to the rapid metabolism of these phthalates in cows. Investigating the metabolism of phthalates and their excretion in urine, milk, etc. was regrettably beyond the scope of this survey. Therefore, it would be interesting if other scientists would do more research about this topic.

In a next phase of this study, the occurrence of phthalates in Belgian milk and dairy products will be investigated at industry and retail level. Since all collected samples will derive from the same Belgian dairy cooperative, phthalate contamination in a whole Belgian milk chain will be discussed.

To date, in Europe, DEHP has been more and more substituted by other plasticisers such as DiNP and DiDP (ECPI, 2010). The phasing out of DEHP was demonstrated in this study by comparing the DEHP concentrations in cow's milk obtained in this and other recent studies with DEHP concentrations from older European studies. Taking into account that DiNP and DiDP have received a tolerable daily intake value by EFSA (2005d; 2005e), it is desirable that future researchers will analyse these compounds in raw cow's milk and other food and feed products as well.

II.4.5 Conclusions

In this survey, the most relevant contamination pathways for eight phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) were explored in raw cow's milk from different Belgian farms. Although DMP, DEP, DnBP, DCHP and DnOP were measured in various feed samples, they were not found in raw cow's milk, which might be due to the rapid metabolism of phthalates in cows. DEHP and to a smaller degree also DiBP and BBP concentrations in raw cow's milk varied across seasons and across farms, which reveals the influence of a seasonal variation in feed composition and the influence of using other feed products, disinfectants, cleaning agents, etc. at the farms. Additionally, phthalate containing contact materials that are being used during cultivation, transport, processing or during the milking process seem to be another important contamination pathway, since phthalate concentrations in manually obtained milk samples differed from concentrations in mechanically obtained milk samples. Furthermore, the results obtained in this survey confirm that the amount of DEHP in European cow's milk has decreased over the last decades.

II.5 Milk 2 - Contamination pathways of phthalates in a contemporary Belgian milk chain

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S. and Sioen, I. (2013). ***Transfer of eight phthalates through the milk chain - A case study.*** *Environment International, 51, 1-7.*

Abstract

This survey determined the levels of eight phthalates – i.e. dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP) – in several Belgian milk and dairy products. Samples were obtained from various farms, a dairy factory and from different shops in order to investigate phthalate contamination "from farm to fork". At several stages in the milk chain, product contamination with phthalates – mostly DiBP, DnBP, BBP and DEHP – was observed. At farm level, the mechanical milking process and the intake of phthalate containing feed by the cattle were found to be possible contamination sources. At industry and retail level, contact materials including packaging materials were additional contamination sources for phthalates in milk and dairy products.

II.5.1 Introduction

Phthalates are one of world's most used groups of plasticisers. Among other things, they are added to plastic polymers (e.g. polyvinyl chloride) to enhance flexibility and can be present in printing inks and lacquers to improve surface adhesion, flexibility and wrinkle resistance. Human exposure to several of these chemical substances is mainly caused via food ingestion (Cao, 2010; ECPI, 2010). Since some phthalates are suspected to cause detrimental effects to human health – e.g. di(2-ethylhexyl) phthalate (DEHP) can disrupt the human endocrine system (Latini et al., 2004) – it is important to know how and to what extent food products are contaminated with phthalates.

In this survey, the contamination of milk and dairy products with phthalates was investigated and this for two important reasons. First, milk and dairy products are an important food group for the (Belgian) population, especially for young children (Vanhauwaert, 2012). Knowing the contents of phthalates in these kinds of food products is an important step when aiming to assess humans' dietary exposure to phthalates. Second, phthalates are lipophilic and therefore tend to concentrate in the lipid phase of foodstuffs. Since dairy products like cream, butter and cheese are high-fat foods, they are expected to be more contaminated with phthalates than low-fat foods (Zhu et al., 2010).

For the last three decades, phthalates have been determined in milk and dairy products by several researchers. Zhu et al. (2010), for instance, mentioned in their review more than ten studies that were conducted between 1986 and 2008 to investigate the presence of phthalates in milk and dairy products. After 2008, researchers still continued analysing phthalates in these types of food (Bradley, 2012; Farajzadeh et al., 2012; Fromme et al., 2011; Li et al., 2011; Yan et al., 2011). In most of the studies, phthalate levels were determined in retail milk and dairy products while only a few surveys reported phthalate concentrations in raw milk and/or in samples collected from dairy factories. However, in order to reduce phthalate contamination in milk (products) if necessary, it is important to know how these compounds enter the milk chain. Therefore, phthalates not only have to be investigated at retail level, but also at other stages of the milk chain.

The objective of this study is to investigate the occurrence of eight phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), DEHP, dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP)) in milk and dairy products according to the principle "from farm to fork". Therefore, samples are collected from various Belgian farms, a dairy factory and from different Belgian shops. Since all obtained samples are deriving from the same dairy cooperative, phthalate contamination in a contemporary Belgian milk chain is discussed – to the authors' knowledge – for the first time.

II.5.2 Material and methods

II.5.2.1 Sample collection

For this study, samples of milk and dairy products were collected at several stages in the milk chain, i.e. at farm, industry and retail level.

At farm level, raw milk samples (n=20) from the cooling tank (point 1 in Figure 8) of five Belgian farms were taken at four different moments: twice during summer 2010 (August-October) and twice during winter 2010-2011 (January-March). All milk samples (4.5% fat on average) were collected in glass bottles (Duran) with polypropylene screw caps, were transported to the laboratory in a cool box and were stored at – 18 °C prior to analysis. More information about the sampling at farm level was recently reported elsewhere (Fierens et al., 2012b).

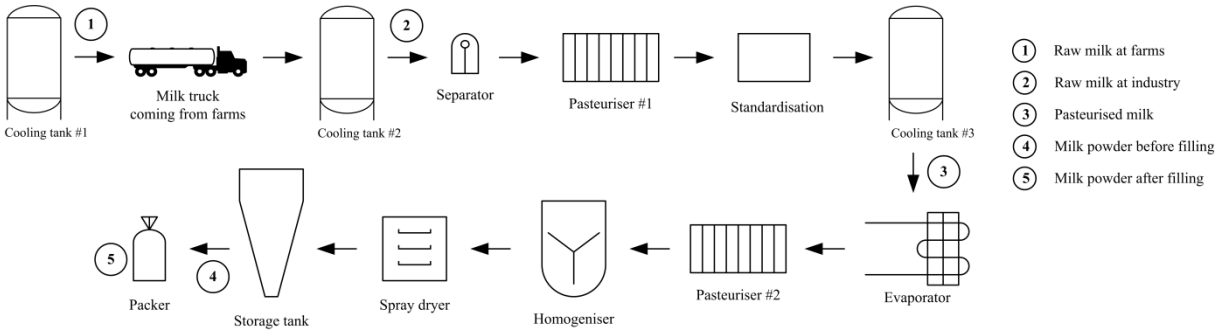

Figure 8: Flow chart of the production process of milk powder. The sampling places considered in this study are indicated with numbers.

A dairy factory that produces milk powder was visited in November 2010 to obtain milk and dairy product samples at industry level. During the production of milk powder – a flow chart of this process is given in Figure 8 – samples were collected at different stages. First, a raw milk sample was taken from the cooling tank at the reception of the factory (point 2 in Figure 8). After the raw milk was purified, separated, pasteurised, standardised and cooled, three samples were gathered from three cooling tanks inside the factory (point 3 in Figure 8). Subsequently, the milk was concentrated on an evaporator and once more pasteurised. The obtained milk concentrate was then homogenised and spray dried until a powder with a moisture content of 3% and a fat content of at least 26% was obtained. Before filling, three samples of this milk powder were taken (point 4 in Figure 8). At the investigated factory, milk powder was packed in can (tinplate; 2-piece with heat-sealed aluminium foil end) or in pouch (multilayer of polyethylene terephthalate, aluminium and polyethylene), both in packages of 400 g. For analysis, three milk powder samples of each packaging type (point 5 in Figure

8) as well as an unused packaging of both materials were collected. At every sampling moment, hands were cleaned with water and no gloves were used. Glass bottles (Duran) of 250 ml with polypropylene screw caps were used to collect all unpacked samples (i.e. raw milk, pasteurised milk and unpacked milk powder). Just like at farm level, all glass bottles were transported to the laboratory in a cool box and stored at – 18 °C prior to analysis. Packed milk powder samples and unused packaging materials were stored at room temperature before analysis.

To investigate phthalate contamination at retail level, samples of milk, butter and cheese were purchased from various Belgian shops between March and September 2010. All milk samples (1.5% fat (low-fat milk), n=5) were packed in Tetra Brik and were from the same brand. One of these samples was bought in winter (March), while the other four samples were purchased in summer (May-September). Four butter samples of two different brands ("A" and "B") were bought (n=2x2). Both butter varieties were packed in an aluminium foil-paper multilayer and contained 82% fat. All cheese samples were hard cheeses and were packed in plastic. From the same brand, fully mature cheese ("cheese A", 33% fat, one year matured, n=2) as well as young cheese ("cheese B", 30% fat, five weeks matured, n=1) were purchased. A third cheese variety ("cheese C", n=1; another brand) was a low-calorie semi-mature cheese with a fat content of 15% and a maturing time of eight weeks. Lastly, samples of milk powder packed in can ("A", n=3) and in pouch ("B", n=3) from the dairy factory were stored for twelve weeks at room temperature in their original packaging to act as retail milk powder (fat content of 28%).

II.5.2.2 Analytical procedure

The analysis of DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP in milk, dairy products and packaging materials was performed according to the analytical procedure for high-fat foods and packaging materials described recently (Fierens et al., 2012c). Briefly, 5 to 80 g of every milk or dairy product sample was extracted with acetone/*n*-hexane (1:1) to obtain at least 0.5 g fat. For the extraction of packaging materials with *n*-hexane followed by a solvent exchange to dichloromethane, 44 cm^2 and 314 cm^2 of unused can and pouch were used, respectively. Subsequently, gel permeation chromatography (GPC) was applied to purify all fat extracts. The GPC system used (Shimadzu) consisted of a HPLC pump (LC-20AT), an autosampler (SIL-20AC), a fraction collector (FRC-10A) and a UV-VIS detector (SPD-20AC). Separation took place on a Waters Envirogel column (19 x 300 mm) packed with styrene divinylbenzene copolymer particles (pore size 100 Å). Dichloromethane acted as mobile phase (flow rate of 4 ml/min). The instrumental analysis of the eight phthalates of interest was performed by means of gas chromatography-low resolution-mass spectrometry with electron impact ionisation (GC-EI-MS; Agilent 5975C inert XL EI/CI MSD with Triple-Axis Detector). Sample injection occurred at 250 °C in splitless mode and phthalates were separated on a DB-XLB column (60 m length, 0.25 mm internal diameter, 0.25 µm film thickness) with a non-polar stationary phase. After 1 min at 50 °C, the temperature of the GC oven increased to 320 °C at 15 °C/min and was then held constant at 320 °C for 15 min. Detection of the different phthalate compounds took place in selected ion monitoring (SIM) mode. For all phthalates except DMP, the dominant product ion *m/z* 149 was used as target ion; for DMP, *m/z* 163 was used (Table 34). As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compounds in a sample had to be within 20% of that observed in a standard solution. Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. The retention times

on the DB-XLB column and the characteristic *m/z* values for these compounds can be found in Table 34 as well.

Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (twelve samples at the most) to reduce the risk of contamination. For high-fat foods (i.e. milk and dairy products), procedural blanks consisted of dichloromethane, to which internal standard was added; for packaging materials, *n*-hexane spiked with internal standard and exchanged to dichloromethane was used. Sunflower oil, to which phthalates were added in a concentration of 250 µg/kg fat, acted as reference sample for the analysis of high-fat food products (sunflower oil without addition of native compounds was analysed as well) and one of the selected packaging samples was applied as a reference sample for the analysis of packaging materials. Therefore, phthalates in a concentration of 30 ng/cm² were added to the extract of the packaging sample. Recoveries varied between 93 and 100% for high-fat foods and between 82 and 99% for packaging materials. Relative standard deviations (RSDs) were less than 14% for each phthalate compound (Fierens et al., 2012c).

Table 34: Retention times, target ions and qualifier ions for the phthalates of interest and their corresponding deuterium-labelled internal standards.

Phthalate compound	Corresponding internal standard	Retention time (min)	Target ion (*m/z*)	Qualifier ion (*m/z*)
DMP	d_4-DMP	13.4	163	194
DEP	d_4-DEP	14.6	149	177
DiBP	d_4-DiBP	16.4	149	223
DnBP	d_4-DnBP	17.1	149	223
BBP	d_4-BBP	19.8	149	206
DEHP	d_4-DEHP	20.6	149	167
DCHP	d_4-DEHP	20.9	149	167
DnOP	d_4-DnOP	22.0	149	279
Internal standards				
d_4-DMP		13.4	167	198
d_4-DEP		14.5	153	181
d_4-DiBP		16.4	153	227
d_4-DnBP		17.1	153	227
d_4-BBP		19.7	153	210
d_4-DEHP		20.6	153	171
d_4-DnOP		22.0	153	283

II.5.2.3 Reporting of results

Phthalate concentrations in milk and dairy products were expressed in micrograms per kilogramme fat (µg/kg fat). To compare with concentrations from other studies (see Section II.5.4.2), the fat based results were converted to µg/kg fresh weight using the fat content mentioned on the packaging or determined experimentally. Levels of phthalates in packaging materials were reported in nanogrammes per square centimetre (ng/cm²).

Due to the omnipresence of phthalates in the (laboratory) environment, limits of quantification (LOQs) strongly depended on the feasible blank concentrations. Therefore, LOQ values were calculated according to the phthalate concentrations detected in the procedural blanks (sum of average blank concentration and six times the standard deviation of replicate procedural blanks). The

LOQs from this survey are listed in Table 35. For milk and dairy products, LOQs varied, depending on the phthalate compound, between 5 and 60 µg/kg fat (based on 28 procedural blanks). The LOQs for the considered packaging materials ranged from 0.001 to 3.3 ng/cm² (based on two procedural blanks).

Table 35: Limits of quantification (LOQs) obtained for every matrix.

Phthalate	Milk and dairy products (µg/kg fat)	Can (ng/cm²)	Pouch (ng/cm²)
DMP	5	0.03	0.003
DEP	20	0.07	0.009
DiBP	15	0.04	0.006
DnBP	15	0.05	0.007
BBP	10	0.01	0.002
DEHP	60	3.3	0.5
DCHP	15	0.02	0.003
DnOP	10	0.01	0.001

II.5.3 Results

An overview of the DiBP, DnBP, BBP and DEHP concentrations determined in milk, dairy products and packaging materials from the investigated milk chain is given in Table 36. DMP, DEP, DCHP and DnOP could generally not be quantified in milk and dairy products and are therefore not mentioned in this table. However, traces of these four compounds were found in the two investigated unused packaging materials: pouch contained measurable levels of DMP, DEP and DCHP (0.01, 0.06 and 0.1 ng/cm², respectively) whereas DnOP could be quantified in can (concentration of 0.2 ng/cm²).

As already reported (Fierens et al., 2012b), raw cow's milk deriving from various Belgian farms contained measurable levels of DEHP and to some extent also DiBP, DnBP and BBP. Differences were noticed between milk collected in summer and winter.

Raw milk collected from cooling tank #2 at the dairy factory only contained DEHP. After purifying, separation, pasteurisation, standardisation and cooling, again only DEHP was detected in milk at a level above the LOQ (based on median concentrations). Furthermore, it should be noticed that the median DEHP concentration increased from 364 µg/kg fat in raw milk to 426 µg/kg fat in pasteurised milk. Once the cooled milk was concentrated, pasteurised, homogenised and spray dried, the median DEHP concentration augmented for a second time, namely from 426 to 478 µg/kg fat. In addition, the obtained milk powder also contained quantifiable levels of DiBP and DnBP. After packaging, increases in DEHP, DnBP, BBP and for canned milk powder also DiBP contents were observed (based on median concentrations). Quantifiable levels of these four phthalates were also determined in the related packaging materials. Comparing phthalate levels in both packaging materials revealed that can contained more phthalates than pouch.

Milk powder still contained DiBP, DnBP, BBP and DEHP when it was stored for twelve weeks at room temperature in their original packaging. The median concentration of DiBP and BBP even increased after storage in can and pouch, respectively. On contrary, the DEHP content in canned milk powder diminished during storage. Levels of the other compounds remained more or less constant during storage.

At retail level, quantifiable levels of DiBP, DEHP and to some extent also DnBP were determined in low-fat milk. With regard to the time of sampling (one sample was bought in winter and the others in summer), a remarkable difference in DEHP content was noticed, namely 312 µg/kg fat in winter compared to 463-535 µg/kg fat in summer (detailed results not shown in Table 36), which is in the same trend as what was observed at farm level. Butter packed in foiled paper only contained DEHP, while retail cheese was also contaminated with DiBP and DnBP.

Table 36: DiBP, DnBP, BBP and DEHP concentrations determined in milk, dairy products and packaging materials from the investigated milk chain. Concentrations in foods are reported in µg/kg fat and concentrations in packaging materials in ng/cm².

Sample type	Sampling time	N	Fat (%)	DiBP		DnBP		BBP		DEHP	
				Min-Max	Median	Min-Max	Median	Min-Max	Median	Min-Max	Median
Farm											
Raw milk cooling tank #1[a]	Aug-Oct 2010 ("summer")	10	4.5[b]	ND-<15	ND	ND-15	ND	ND-16	ND	ND-788	390
Raw milk cooling tank #1[a]	Jan-March 2011 ("winter")	10	4.5[b]	ND-52	23	ND-<15	<15	<10-21	<10	201-500	280
Industry											
Raw milk cooling tank #2	Nov 2010	1	NA	<15	<15	ND	ND	ND	ND	364	364
Pasteurised milk cooling tank #3	Nov 2010	3	NA	<15	<15	ND-<15	ND	ND-14	ND	332-443	426
Milk powder before filling	Nov 2010	3	27.5[b]	24-90	32	24-35	28	ND	ND	462-489	478
Milk powder after filling (can)	Nov 2010	3	28	49-64	56	50-54	52	11-13	12	584-634	630
Milk powder after filling (pouch)	Nov 2010	3	28	31-33	31	57-64	60	43-61	53	409-609	523
Unused packaging (can)	Nov 2010	1	-	2.6	2.6	0.5	0.5	0.3	0.3	14	14
Unused packaging (pouch)	Nov 2010	1	-	0.7	0.7	0.1	0.1	0.1	0.1	2.7	2.7
Retail											
Milk powder A (can)[c]	Feb 2011	3	28	62-85	75	51-58	53	12-13	12	524-591	566
Milk powder B (pouch)[c]	Feb 2011	3	28	23-30	27	54-98	56	41-68	65	519-534	526
Milk (Tetra Brik)	March-Sept 2010	5[d]	1.5	<15-28	18	<15-34	<15	ND-<10	ND	312-535	463
Butter A (foiled paper)	June 2010	2	82	ND	ND	ND	ND	ND	ND	241-248	245
Butter B (foiled paper)	June 2010	2	82	ND	ND	ND	ND	ND	ND	350-3,350	350
Cheese A (fully mature; plastic)	Sept 2010	2	33	20-23	21	<15	<15	ND	ND	360-412	386
Cheese B (young; plastic)	Sept 2010	1	30	22	22	45	45	ND	ND	731	731
Cheese C (semi-mature; plastic)	Sept 2010	1	15	36	36	100	100	<10	<10	530	530

N: number of samples; NA: not available; ND: not detected; <Value: detected, but lower than LOQ value; -: not applicable; [a] Results recently described in detail in Fierens et al. (2012b); [b] Average fat content; [c] Milk powder from dairy factory after twelve weeks of storage at room temperature in original packaging; [d] One winter sample (purchased in March) and four summer samples (purchased between May and September).

II.5.4 Discussion

In this survey, levels of eight phthalates were determined in several Belgian milk and dairy products. Samples were collected from farms, a dairy factory and were purchased from various shops. Since all samples were deriving from the same dairy cooperative, phthalate contamination in an entire milk chain can be discussed.

II.5.4.1 Phthalate contamination in the milk chain

Phthalate contamination at farm level is recently discussed in detail by Fierens et al. (2012b). Briefly, of the eight phthalates considered, only DiBP, DnBP, BBP and DEHP were present in raw cow's milk (point 1 in Figure 8). At the investigated farms, raw milk seemed to be contaminated with DiBP and DEHP due to the ingestion of contaminated feed (i.e. silage and for DEHP also pasture). Contact materials used during the mechanical milking process were responsible for the occurrence of BBP and DEHP in milk. Additionally, differences in phthalate levels were noticed between raw cow's milk sampled in winter and in summer. For instance, summer milk contained generally more DEHP than winter milk.

Almost no extra phthalate contamination occurred during the transportation of milk from the cooling tanks of the farms to the cooling tank of the dairy factory (points 1 and 2 in Figure 8), since comparable phthalate contents in raw milk at the farms and the factory were determined (Table 36). This was also concluded in a Norwegian study (Sharman et al., 1994). After separation, pasteurisation, standardisation and cooling (point 3 in Figure 8), the DEHP content in milk augmented from 364 to 426 µg/kg fat (median level). This increase was most likely due to DEHP containing food contact materials (e.g. tubings and sealants) used during processing (Cao, 2010). Moreover, it is known that phthalate migration is accelerated by heat (Castle, 2007), so DEHP migration into the milk might have been facilitated during pasteurisation. Besides DEHP, also DiBP and DnBP contents raised after milk was concentrated to obtain milk powder (point 4 in Figure 8). Migration from contact materials almost certainly took place and was probably improved during the second pasteurisation step. Considering that milk powder has a large contact surface might have enhanced the migration of DiBP, DnBP and DEHP as well (Castle, 2007; Page and Lacroix, 1995). After the packaging step (point 5 in Figure 8), DEHP levels raised considerably in canned milk powder, while an overlap in concentration range was observed for milk powder packed in pouches. DiBP concentrations in packed milk powder were in line with those in unpacked powder, while levels of DnBP increased after packaging. Also BBP was detected in quantifiable levels, especially in milk powder packed in pouches. Since these four phthalates were also found in the two related packaging materials, packaging can be seen as an additional – but definitely not as the sole – contamination source at the factory. This is very reasonable, as it is commonly known that phthalates are used in printing inks, adhesives, epoxy resins, etc. of several types of packaging materials (Bradley, 2012; Cao, 2010). On contrary, DiBP, DnBP, BBP and DEHP contents did not increase after milk powder was being stored for twelve weeks at room temperature in the original packaging (contents were measured after zero, three, six, nine and twelve weeks of storage at room temperature; results not shown). Thus, during storage at room temperature, phthalate migration from packaging into milk powder did not seem to occur. This is not surprising, since migration at room temperature is rather low, especially for dry solid foods (Castle, 2007).

Considering retail milk, DMP, DnBP, DiBP and DEHP were detected in levels above the LOQ in one, two, four and five samples, respectively (Table 36; result for DMP, i.e. 7 µg/kg fat, is not shown in this table). Since four of the samples were purchased in summer, phthalate levels in retail milk were compared with concentrations in raw summer milk to decide if contamination occurred in the milk chain. Although the concentrations in one and two samples were higher than the concentration ranges observed at farm level, median levels of DMP and DnBP in retail milk, respectively, were similar to those in raw milk. DMP and DnBP contamination did thus generally not occur during the production of retail milk. On contrary, DiBP contents increased from not detected in raw milk to 18 µg/kg fat in retail low-fat milk (based on medians). Since concentration ranges did not overlap, DiBP contamination almost certainly took place somewhere in the milk chain. Although the median DEHP content in milk increased with 73 µg/kg fat, DEHP concentration ranges overlapped, so it is uncertain if DEHP contamination really occurred during processing. Notwithstanding, it should be noticed that the DEHP content in retail milk bought in winter was remarkably lower than in summer. This confirms the seasonal influence on DEHP concentrations observed in cow's milk at farm level (Fierens et al., 2012b). Regarding the other retail samples, butter packed in foiled paper only contained DEHP, of which the concentrations were in line with the levels determined in raw summer milk. The same phenomenon was observed for DEHP in cheese: ranges at farm and retail level were comparable. Even though, it should be mentioned that the DEHP contents in the three considered cheeses differed. In fact, a relationship with the time of maturing was observed: the longer the cheese was matured, the lower the DEHP content seemed to be, which might be owing to the fact that DEHP possibly degraded during maturing. On contrary, with the exception of DnBP in cheese A, concentrations of DiBP and DnBP augmented from raw milk until retail cheese, which may indicate that DiBP and DnBP contamination took place.

Since phthalates are lipophilic, it is assumed that high-fat foods contain more phthalates than low-fat food products (Zhu et al., 2010). Various research groups (Page and Lacroix, 1995; Sharman et al., 1994; Tsumura et al., 2002b) reported that there is a positive relationship between the fat content of a dairy product and the DEHP content in that product. In this study, the same conclusion was made for the investigated retail samples (Table 37).

II.5.4.2 Comparison with other studies

Levels of the eight phthalates investigated in Belgian raw cow's milk were recently compared with concentrations from other surveys (Fierens et al., 2012b). In general, Belgian concentrations were similar to levels reported in other recent (from 2001 until now) European studies (Cousins and Mackay, 2001; Sorensen, 2006). In contrast, higher phthalate concentrations were noticed in older European (Castle et al., 1990; Sharman et al., 1994) and in recent non-European surveys (Feng et al., 2005; Kim et al., 2009). The European decline in these phthalate levels is most likely due to the fact that the European legislation concerning the use of phthalates such as DnBP and DEHP has been tightened (Official Journal of the European Union, 2005; SCCP, 2007) and that the investigated phthalates like DEHP have been more and more substituted by other plasticisers (ECPI, 2010). For instance, according to Wordsworth (2007), differences between phthalate contents in European and non-European milk are almost certainly due to discrepancies in regulations between countries.

Regarding phthalate contamination at industry level, only one rather old survey could be found, in which comparable research was done. In that study, the occurrence of DEHP in Norwegian milk and

cream was investigated during collection, transportation and packaging (Sharman et al., 1994). At all stages, DEHP contents in Norwegian samples were much higher compared to related Belgian ones. As discussed in the previous section, this contrast is most likely the result of a more strict legislation and a declined use of DEHP in Europe during the last decade.

An overview of phthalate concentrations in retail milk and dairy products reported in several studies (conducted after 2000) is given in Table 37. Concerning retail milk, levels of DMP, DEP, BBP, DCHP and DnOP were generally the same in all surveys. However, it should be noticed that remarkably high median concentrations of DMP and DEP were observed in milk from China (Li et al., 2011) and Spain (Casajuana and Lacorte, 2004), respectively. Spanish (Casajuana and Lacorte, 2004), Chinese (Li et al., 2011) and Iranian milk (Farajzadeh et al., 2012) contained more DnBP than milk investigated by other researchers. DiBP was only measurable – in low concentrations – in some Belgian milk samples (Fierens et al., 2012c and this survey). As also observed in raw cow's milk (Fierens et al., 2012b), non-European retail milk seemed to be more contaminated with DEHP than that of European milk.

Regarding milk powder, Table 37 shows that similar concentrations of DMP, DEP, DCHP and DnOP were observed in all studies (except for DMP and DEP in the study of Casajuana et al. (2004)). On contrary, milk powder investigated in this survey usually contained more DiBP, DnBP, BBP and DEHP. This observation is probably owing to the fact that in the other studies, reconstituted milk powder – i.e. milk powder dissolved in water – instead of "dry" milk powder was investigated. Since dilution factors were lacking in the mentioned surveys, back-calculations to initially weight could not be done, which makes a comparison very hard.

Butter was more contaminated with DEHP in Japan (Tsumura et al., 2002b) than in other countries (Table 37). On the other hand, higher contents of DnBP and BBP were reported by Peters (2006). Since this researcher only investigated one sample, this conclusion should be taken with caution. Levels of DMP, DEP, DiBP, DCHP and DnOP were more or less the same in every survey.

With the exception of DEP in one British cheese sample (Bradley, 2012), DMP, DEP, DCHP and DnOP were generally not detected in retail cheese (Table 37). For the other phthalates, larger concentration ranges were observed by Peters (2006) and by Pfördt (2004).

For all retail products, the conclusion can be made that phthalate levels sometimes varied considerably. Mostly, differences in phthalate contents can be explained by discrepancies in chemicals' legislations between countries (Wordsworth, 2007; Yano et al., 2005). Another reason for this variation might be the use of different food packaging materials. Milk, for instance, seemed to contain more DnBP and DEHP when it is packed in polyethylene than in Tetra Brik (Casajuana and Lacorte, 2004; Farajzadeh et al., 2012). Furthermore, butter wrapped in wash coated paper would also be more sensitive to contain these two phthalates (Page and Lacroix, 1992).

Table 37: Overview of phthalate concentrations (min-max (median)) in retail milk and dairy products obtained in several studies.

Study	Country	N	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
						In µg/kg fresh weight				
Milk										
Tsumura et al., 2002	Japan	3	-	-	-	ND	ND	63-100 (64)	-	-
Casajuana et al., 2004	Spain	4	1.0-1.8 (1.2)	37-85 (72)	-	7.3-50 (25)	1.1-2.9 (2.1)	15-27 (24)	-	-
Peters, 2006	Europe	1	ND	ND	ND	ND	ND	ND	-	-
Sorensen, 2006	Denmark	4	-	-	-	<9	<4	13-27	-	-
Li et al., 2011	China	4	ND-62 (55)	ND-35 (ND)	-	113-131 (124)	ND-24 (ND)	-	ND-51 (ND)	ND-98 (ND)
Yan et al., 2011	China	5	ND-6.4 (ND)	ND	-	ND-5.2 (3.6)	ND	-	-	ND
Bradley, 2012	UK	6	ND	ND	ND	ND	ND	ND-109 (ND)	ND	ND
Farajzadeh et al., 2012	Iran	6	ND	ND	ND	ND-79 (73)	-	ND-201 (191)	-	-
Fierens et al., 2012c	Belgium	8	ND	ND	ND-0.7 (ND)	ND-0.8 (ND)	ND	7.8-20 (14)	ND-4.6 (ND)	ND
This study	Belgium	5	ND-0.1 (ND)	ND	<0.2-0.4 (0.3)	<0.2-0.5 (<0.2)	ND-<0.2 (ND)	4.7-8.0 (6.9)	ND-<0.2 (ND)	ND-<0.2 (ND)
Milk powder										
Casajuana et al., 2004	Spain	1[a]	1.4	76	-	18	1.2	21	-	-
Yano et al., 2005	World	27[a]	-	-	-	15-77	-	34-281	-	-
Sorensen, 2006	World	6[a]	-	-	-	<9	<4	37-138	-	-
Fromme et al., 2011	Germany	4[a]	ND	ND	1.6-4.9 (3.6)[b]	1.7-5.5 (3.8)[b]	ND	9.3-36 (20)[b]	ND	ND
Bradley, 2012	UK	6[a]	ND	ND	ND-13 (ND)	ND	ND	ND-125 (ND)	ND	ND
Fierens et al., 2012c	Belgium	3[a]	ND-0.2 (ND)	ND-0.2 (ND)	2.7-4.9 (3.6)	1.7-6.6 (2.5)	1.9-16 (16)	37-62 (42)	ND-1.8 (0.8)	0.3-3.0 (1.1)
This study	Belgium	6	ND	ND	6.4-24 (13)	14.27 (15)	3.2-19 (7.4)	145-166 (148)	ND-<4.2 (ND)	ND
Butter										
Tsumura et al., 2002	Japan	3	-	-	-	ND	ND-56 (ND)	1,020-2,830 (1,400)	-	-
Peters, 2006	Europe	1	ND	5.6	ND	132	17	770	-	-
Bradley, 2012	UK	4	ND	ND	ND	ND	ND	ND-2,592 (ND)	ND	ND
Fierens et al., 2012c	Belgium	3	ND	ND	ND-12 (ND)	ND	ND-11 (ND)	125-508 (390)	ND	ND
This study	Belgium	4	ND	ND-<16 (ND)	ND	ND	ND	198-287 (245)	ND	ND-<8.2 (ND)
Cheese										
Tsumura et al., 2002	Japan	3	-	-	-	ND	<8-8 (<8)	330-570 (350)	-	-
Pfördt, 2004	Germany	10	-	-	<50-730 (<50)	<50-70 (<50)	-	120-920 (330)	-	-
Peters, 2006	Europe	5	ND	ND	ND-4,400 (ND)	ND-200 (76)	ND-50 (21)	ND-3,000 (210)	-	-
Bradley, 2012	UK	1	ND	272	ND	ND	ND	366	ND	ND
Fierens et al., 2012c	Belgium	21	ND	ND-5.3 (ND)	ND-116 (6.2)	ND-54 (4.6)	ND-8.2 (ND)	31-743 (148)	ND-42 (ND)	ND-3.7 (ND)
This study	Belgium	4	ND	ND	5.5-7.5 (6.5)	<5-15 (<5)	ND-<1.5 (ND)	80-219 (127)	ND	ND-<3.3 (ND)

N: number of samples; ND: not detected; <Value: detected, but lower than LOQ value; -: not investigated; [a] (Reconstituted) infant formula; [b] Average instead of median concentration.

II.5.4.3 Limitations of the study

To the authors' knowledge, this survey discusses the occurrence of eight phthalates in a contemporary Belgian milk chain for the first time. As for every study conducted, also this survey involved some limitations. For instance, since phthalates are ubiquitous, several precautionary measures had to be taken to reduce the risk of contamination during sample preparation and analysis. Consequently, phthalate analyses are time-consuming, which definitely limited the number of samples that could be examined. In this study, the decision was made to investigate one type of dairy product, i.e. milk powder, in depth by collecting samples of milk (powder) at several stages during the production process (incl. storage). By comparing phthalate levels determined at the various stages, contamination sources could be revealed (e.g. pasteurisation). Unfortunately, due to the limited number of samples that were collected at every stage of the production process (i.e. three at most), this study lacked some power to perform statistical tests. This made that the increases in phthalate concentrations that were noticed (e.g. of DnBP in milk powder before and after filling) could not be confirmed statistically. Furthermore, milk, cheese and butter samples were only collected in the beginning and at the end of the milk chain. For these products, specific contamination sources could not be determined. Moreover, due to long production times of some of the products (e.g. the maturing of cheese A took about a year), it is possible that present phthalates could already have been degraded. To confirm or refute this hypothesis, it would be interesting to analyse phthalates' degradation products during production processes and storage in the future as well, because now, it is hard to demonstrate that phthalate contamination really took place. Overall, more research about the occurrence of phthalates in foods is definitely desirable, especially at industry level.

II.5.5 Conclusions

This survey discussed the occurrence of eight phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) in a Belgian milk chain. Therefore, milk and dairy product samples were collected at farm, industry and retail level. At the considered farms, BBP and DEHP migrated into raw cow's milk during the mechanical milking process. Ingestion of contaminated feed additionally contaminated this raw milk with DiBP and DEHP (Fierens et al., 2012b). When milk was transported from the farms to the dairy factory, almost no extra phthalate contamination took place. Contact materials including packaging materials increased the levels of DiBP, DnBP, BBP and DEHP in milk (powder) at the dairy factory. Comparing phthalate concentrations in retail products with levels in raw cow's milk revealed that, somewhere in the milk chain, milk and cheese were extra contaminated with DiBP and DnBP.

II.6 Heat – Effect of home-cooking on the levels of phthalates in foods

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Abstract

Food products can be contaminated with toxic compounds via the environment. Another possibility of food contamination is that toxicants are generated in foods or that chemicals migrate from food contact materials into foods during processing. In this study, the effect of cooking at home on the levels of phthalates – world's most used group of plasticisers – in various food types (starchy products, vegetables and meat and fish) was examined. Eight compounds were considered, namely dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP). Food products were analysed before as well as after cooking (boiling, steaming, (deep-)frying or grilling). In general, phthalate concentrations in foods declined after cooking, except in vegetables, where almost no effect was seen. Several factors influenced the degree of this decline (e.g. weight difference, fat uptake, etc.). Of all phthalates, DEHP, DiBP and BBP were affected the most. In conclusion, cooking at home definitely affected phthalate concentrations in foods and thus needs to be considered in order to correctly assess humans' dietary exposure to these contaminants.

II.6.1 Introduction

Humans are exposed to thousands of different chemicals each day. The origin of these chemical substances can be either natural or man-made. Once released into the environment, chemicals can pollute air, water, soil and, as a consequence, also food (WHO, 2000b). Another possibility of food contamination is that toxicants are generated during processing. Furan, for example, is formed in tinned foods during heating (Roberts et al., 2008) and acrylamide is formed in heat processed carbohydrate rich foods as a side reaction of the Maillard reaction, i.e. a non-enzymatic browning reaction between amino acids and reducing sugars (Medeiros Vinci et al., 2012). Chemical substances can also migrate from food contact materials into food (Castle, 2007).

Plenty of researchers have investigated the effect of cooking on chemical concentrations in foods. Investigators considered a.o. the effect of processing and cooking on polybrominated diphenyl ethers, polychlorinated biphenyls, hexachlorobenzene, polycyclic aromatic hydrocarbons, furan, pesticides, arsenic, cadmium, mercury and lead (Bayen et al., 2005; Chavarri et al., 2005; Perelló et al., 2008; 2009; Roberts et al., 2008). However, concerning the influence of cooking on phthalates – one of world's most used group of plasticisers – little information is known. In fact, only one study could be found, a Japanese survey, in which the effect of several cooking methods on the concentration of di(2-ethylhexyl) phthalate (DEHP) in chicken eggs, liver and meat was carried out (Ishida, 1993).

Knowing the impact of cooking on phthalate concentrations in food is important for several reasons. Firstly, food products like starchy products and meat are mainly eaten cooked. Thus, for intake studies, it is rather irrelevant to determine phthalate levels in these kinds of products on a raw basis, since they are not consumed in that way. Furthermore, phthalates can be present in coatings of

cooking materials and thus can migrate from cookware into food during the preparation process (Bradley et al., 2007). Considering the fact that this migration is accelerated by heat (Castle, 2007), makes the risk of contamination even higher. Lastly, food intake is by far the most important human exposure pathway for many phthalate compounds (Clark et al., 2003a). So, in order to be able to correctly assess the population's dietary exposure to these contaminants, it is important to consider the effect of cooking.

This study investigates the influence of cooking at home on phthalate concentrations in various food products. Eight compounds are considered, namely dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), DEHP, dicyclohexyl phthalate (DCHP) and di-*n*-octyl phthalate (DnOP). Examined foodstuffs include starchy products, vegetables, meat and fish. Various preparation methods are carried out, i.e. boiling, steaming, (deep-)frying and grilling.

II.6.2 Material and methods

II.6.2.1 Sample collection

For this study, 15 food samples were purchased from various Belgian shops between February and April 2011. Samples included potato (a waxy cultivar ("cultivar 1"), a cultivar specifically for chips ("cultivar 2") and pre-fried frozen chips), rice (loose and boil-in-bag, same brand), pasta (two white varieties and one wholemeal variety), vegetables (carrot, cauliflower, onion and paprika (red, yellow and green)), meat (minced meat and pork chop) and fish (salmon). Potato cultivar 1 was prepacked in a plastic mesh bag; potato cultivar 2 and pre-fried frozen chips in ordinary plastic bags. Loose rice was only prepacked in cardboard, while the other rice variety ("boil-in-bag", same brand) also consisted of plastic boiling bags. The packaging of wholemeal pasta and one white pasta variety (same brand) was cardboard; the other white pasta variety was prepacked in plastic. Of all vegetables investigated, only paprika was prepacked (in plastic). Minced meat and pork chop were packed at the shop in duplex paper (paper with polyethylene lining) using a plastic spoon and plastic gloves, respectively. Finally, fresh salmon was packed, also at the shop, in a sealed bag (multilayer of aluminium, paper and polyethylene) using plastic gloves. An overview of all purchased food samples including their packaging types is given in Table 38.

Table 38: Overview of the investigated food samples and their packaging types, applied cooking processes, food weights before and after cooking, amounts of cooking media used and applied cooking times. For each cooking process, one food sample was studied, of which two subsamples were taken for analysis.

Food	Packaging	Cooking process	Food weight before (g)	Food weight after (g)	Cooking medium (amount)	Cooking time (min)
Potato (cultivar 1)	Plastic mesh bag	Boiling	309.4	328.5	Water (754 ml)	18.5
		Steaming	234.6	237.9	Water (910 ml)	18.0
		Frying 1[a]	65.8	42.3	Margarine (26 g)	11.3
Potato (cultivar 2)	Plastic bag	Pre-frying	474.6	308.6	Vegetable oil (2,000 ml)	5.0
		Deep-frying	178.2	159.5	Vegetable oil (2,000 ml)	3.0
Chips[b]	Plastic bag	Deep-frying	139.6	99.3	Vegetable oil (2,000 ml)	4.0
Rice (loose)	Cardboard	Boiling	64.3	248.7	Water (996 ml)	12.0
Rice (boil-in-bag)	Cardboard with plastic boiling bags	Boiling	125.3	384.0	Water (997 ml)	12.0
Pasta (white)	Plastic	Boiling	29.8	85.3	Water (1,000 ml)	6.0
Pasta (white)	Cardboard	Boiling	104.5	297.6	Water (1,000 ml)	7.5
Pasta (wholemeal)	Cardboard	Boiling	23.5	73.1	Water (1,000 ml)	9.0
Carrot	-	Boiling	59.6	58.2	Water (938 ml)	12.0
		Steaming	69.6	64.7	Water (875 ml)	13.0
Cauliflower	-	Boiling	243.3	268.0	Water (1,633 ml)	10.0
		Steaming	151.6	159.8	Water (829 ml)	13.0
Onion	-	Frying 1	119.4	87.4	Margarine (10 g)	5.5
Paprika	Plastic	Frying 1	67.6	42.2	Margarine (16 g)	6.0
Minced meat	Duplex paper[c]	Frying 1	73.2	55.3	Margarine (20 g)	3.5
		Frying 2	78.6	62.0	-	4.0
Pork chop	Duplex paper[c]	Frying 1	55.0	45.1	Margarine (22 g)	7.0
		Frying 2	72.9	64.3	-	6.5
Salmon	Sealed bag[d]	Frying 1	37.1	28.6	Margarine (21 g)	5.5
		Frying 2	43.8	40.1	-	4.0
		Grilling 1	38.6	35.8	-	8.0
		Grilling 2	38.4	37.1	-	17.0

Frying 1: Frying in a frying pan with margarine; Frying 2: Frying in a non-stick frying pan without margarine; Grilling 1: Grilling in oven without aluminium foil; Grilling 2: Grilling in oven with aluminium foil ("en papillote"); [a] Prior to frying, potato cultivar 1 was boiled; [b] Purchased pre-fried frozen chips; [c] Paper with polyethylene lining; [d] Multilayer of aluminium, paper and polyethylene.

II.6.2.2 Cooking processes and cooking materials

All food products were prepared and cooked in a way an ordinary (Belgian) household would do (a schematic representation of this can be found in Figure 9). Thus, before cooking, potato cultivars, carrots and cauliflower were peeled (i.e. inedible parts were removed), washed with tap water and cut into pieces. Paprika was washed with tap water and cut into pieces, while onion was peeled and shred prior to cooking. For boiling, a stainless steel pot filled with tap water was used. The same pot filled with tap water and a stainless steel steamer insert was utilised to steam some food products. For food samples that were fried, a stainless steel frying pan with margarine ("frying 1") and a non-stick frying pan without margarine ("frying 2") were employed. All above mentioned cooking processes took place on an induction cooker. Furthermore, a deep fryer filled with vegetable frying oil was utilised for pre-frying (at 160 °C) and deep-frying (at 180 °C). Lastly, grilling occurred in a microwave with grill function without ("grilling 1") or with ("grilling 2") aluminium foil wrapped around the food. The cooking media used – i.e. tap water, margarine and vegetable frying oil – were tested before use on the presence of phthalates. In all media, phthalate compounds were not detected or concentrations were below the limit of quantification (LOQ; results not shown). Prior to frying, potato cultivar 1 was boiled. After every deep-frying process, new vegetable frying oil was used. All the cooking processes that were applied on the investigated food samples are shown in Table 38. The weights of the food samples before and after cooking as well as amounts of cooking media used and applied cooking times can also be consulted in this table. After every cooking process, cooking materials were cleaned with hot water and detergent to avoid cross-infection.

II.6.2.3 Analytical procedure

DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP were analysed in the various foodstuffs before (i.e. after washing, peeling, etc.) and after cooking according to the analytical procedure described recently (Fierens et al., 2012c). All foods were analysed in duplicate, which means that, before and after each cooking process, two subsamples were taken to prepare and analyse separately (Figure 9). For the sample preparation, a distinction was made between high-fat foods (all minced meat, salmon, fried, pre-fried and deep-fried samples, except for fried onion and pork chop) and low-fat foodstuffs. Briefly, 5-20 g of every high-fat food sample was extracted to obtain at least 0.5 g fat. For the extraction of low-fat foods, 10 g was used. Subsequently, all extracts were purified via gel permeation chromatography (GPC) and the phthalates of interest were analysed by means of gas chromatography-low resolution-mass spectrometry with electron impact ionisation (GC-EI-MS). Detection of the different phthalate compounds took place in selected ion monitoring (SIM) mode. For all phthalates except DMP, the dominant product ion *m/z* 149 was used as target ion; for DMP, *m/z* 163 was used. As a criterion for a positive identification, the ion ratio (qualifier/target) of the phthalate compounds in a sample had to be within 20% of that observed in a standard solution. Quantification of the phthalates of interest occurred in relation to the corresponding deuterium-labelled internal standards. Each analytical sequence was composed of two procedural blanks, several solvent blanks, calibration standards, a reference sample and a limited amount of samples (twelve samples at the most) to reduce the risk of contamination. The analytical procedure was well reproducible as recoveries varied between 93 and 100% for high-fat foods and between 88% and 102% for low-fat foods. Additionally, relative standard deviations (RSDs) and measurement uncertainties were lower than 11% and 30% for each phthalate compound, respectively.

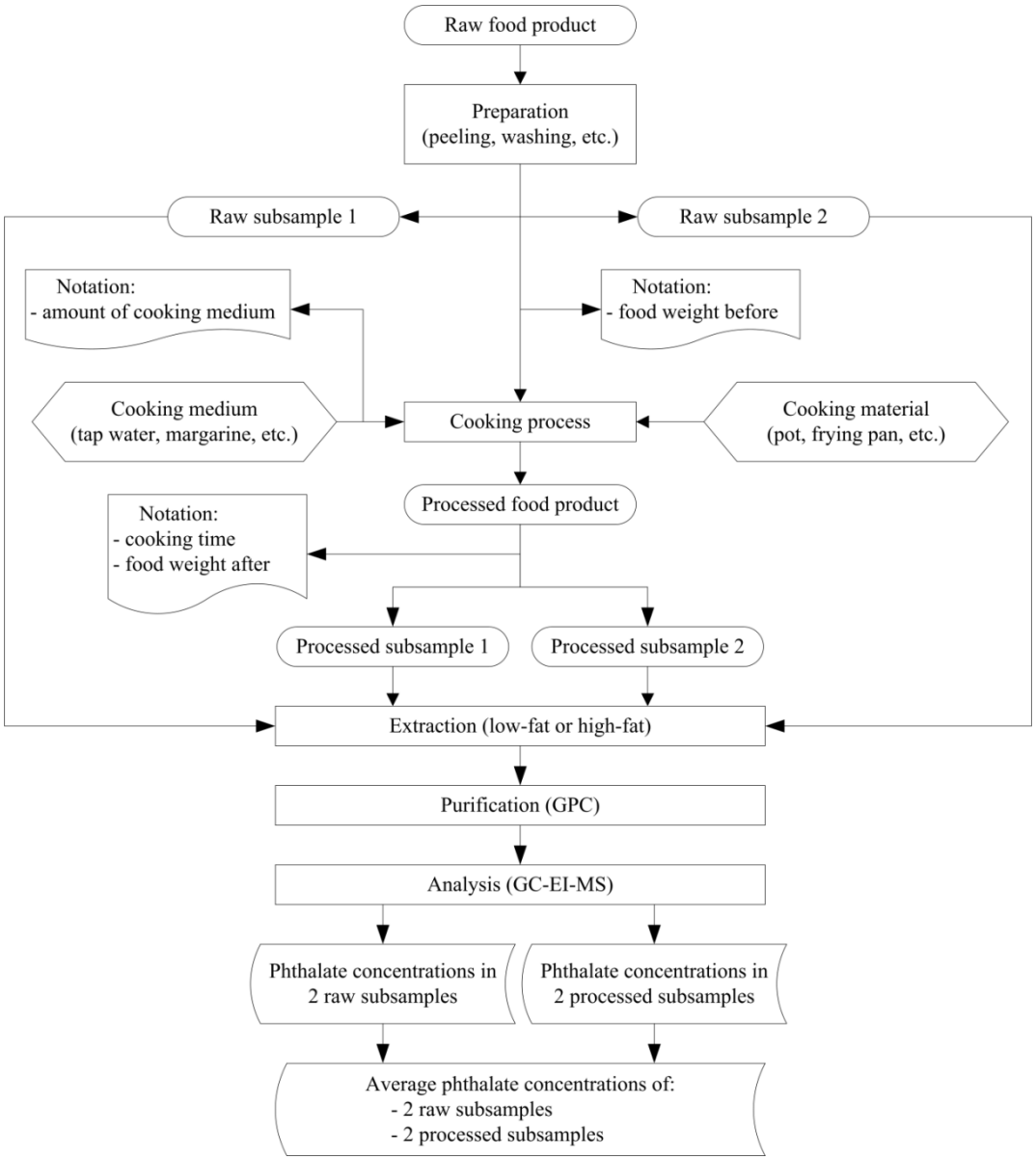

Figure 9: Schematic representation of the experimental design. GPC: gel permeation chromatography; GC-EI-MS: gas chromatography-low resolution-mass spectrometry with electron impact ionisation.

II.6.2.4 Reporting of results

Phthalate concentrations were expressed in micrograms per kilogram product (µg/kg product). For high-fat food products, initial concentrations were expressed in µg/kg fat. This means that a conversion to µg/kg product had to be made by using the (experimentally determined) fat content of the corresponding foods. Since every sample was analysed in duplicate, average phthalate concentrations were calculated.

Because phthalates are ubiquitous, LOQs strongly depended on the feasible blank concentrations. Therefore, for each phthalate compound, LOQ calculations were based on the concentrations detected in the procedural blanks – i.e. the sum of the average blank concentration and six times the standard deviation of replicate procedural blank measurements. In this study, LOQs varied,

depending on the phthalate compound, between 5 and 100 µg/kg fat (0.25 and 80 µg/kg product, respectively) for high-fat food samples (based on 8 procedural blanks) and between 0.05 and 7.25 µg/kg product for low-fat food samples (based on 14 procedural blanks).

II.6.2.5 Statistical analyses

Statistical analyses were carried out using R version 2.15.1 (R Development Core Team, 2011). Wilcoxon signed-rank tests were used to assess the effect of cooking on phthalate concentrations and were only performed if the number of paired samples was more than five (five raw and five corresponding processed samples). Statistical significance was based on the level of $p < 0.05$. Concentrations of phthalates, which were not detected, were set at zero. For phthalate compounds, which were detected in levels below the LOQ, concentrations were replaced by half of the LOQ.

II.6.3 Results

Figure 10 shows the number of positive samples (>LOQ, in %) for each phthalate compound in all investigated food samples before ("raw") and after ("processed") cooking. From the figure, it can be noticed that DMP, DnBP, DCHP and DnOP were only quantifiable in ≤50% of the samples. On contrary, DEHP was present in all considered raw foodstuffs, although this percentage decreased to 65.4% after cooking. Also for the other compounds, the number of positive samples declined after processing, except for DMP and DiBP, of which the percentages raised from 28.6% and 78.6% to 34.6% and 88.5%, respectively.

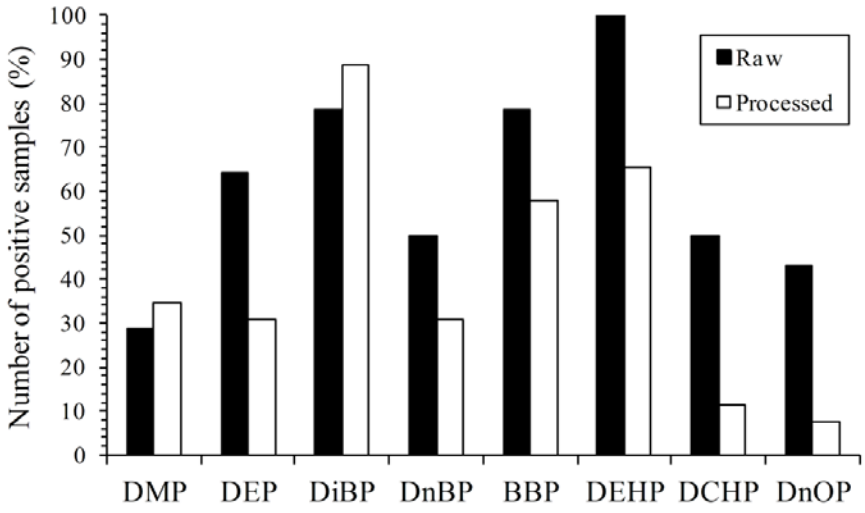

Figure 10: Number of positive samples (>LOQ, in %) for each phthalate compound in all food products before ("raw") and after ("processed") cooking.

Phthalate concentrations in raw and processed starchy products – i.e. potatoes, rice and pasta – are given in Table 39. With the exception of DMP and DEP in wholemeal pasta packed in cardboard, concentrations of all compounds in starchy products reduced after boiling. Levels of BBP and DEHP decreased significantly ($p = 0.036$ and $p = 0.031$, respectively). Although not significant, the same trend was observed for DEP ($p = 0.281$), DiBP ($p = 0.059$) and DnBP ($p = 0.074$). Steaming caused a small augmentation of the DiBP concentration in potatoes (cultivar 1), while the level of DEHP

declined and other phthalates remained not quantifiable or not detected. Frying of this potato cultivar (potatoes were first boiled) in a stainless steel pan with margarine not only increased the concentration of DiBP, but also that of DnBP, BBP and DEHP. Furthermore, potatoes (cultivar 2 and purchased pre-fried frozen chips) deep-fried in vegetable oil contained less phthalates than the uncooked potato cultivars. This conclusion was made with the exception of DiBP, of which the concentrations slightly increased after deep-frying.

Table 39: Phthalate concentrations (average of two subsamples) in raw and processed starchy products (in µg/kg product).

Food	Type	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Potato (cultivar 1)	Raw	<0.05	ND	ND	ND	<0.20	13.8	ND	<0.15
	Boiled	ND	ND	ND	<2.30	ND	ND	ND	ND
	Steamed	ND	ND	1.63	ND	<0.20	<7.25	ND	ND
	Fried 1[a]	<1.00	<1.00	5.69	4.21	1.49	49.8	ND	ND
Potato (cultivar 2)	Raw	ND	<0.15	<1.00	ND	0.37	69.6	<0.10	ND
	Pre-fried	ND	ND	0.53	ND	ND	44.4	ND	ND
	Deep-fried	ND	ND	0.83	ND	ND	<15	ND	ND
Chips	Pre-fried[b]	0.08	0.32	1.77	2.56	0.83	125	<0.10	ND
	Deep-fried	ND	ND	2.09	<2.25	0.76	109	ND	ND
Rice (loose)	Raw	0.07	0.78	383	28.0	21.3	54.3	0.61	0.24
	Boiled	0.06	<0.15	25.3	<2.30	2.02	ND	0.12	ND
Rice (boil-in-bag)	Raw	<0.05	0.90	21.4	2.94	3.16	25.5	0.10	ND
	Boiled	<0.05	0.20	3.27	ND	0.43	ND	<0.10	ND
Pasta (white, PL)	Raw	ND	<0.15	2.80	<2.30	<0.20	21.1	<0.10	0.18
	Boiled	<0.05	ND	<1.00	ND	ND	ND	ND	ND
Pasta (white, CB)	Raw	<0.05	0.36	53.3	13.3	0.23	41.1	0.39	0.35
	Boiled	<0.05	ND	9.59	<2.30	ND	ND	ND	ND
Pasta (WM, CB)	Raw	<0.05	0.30	28.3	3.78	0.25	14.6	0.21	0.19
	Boiled	0.10	0.91	6.39	ND	<0.20	<7.25	ND	<0.15

ND: not detected; <value: detected, but lower than LOQ value; PL : Plastic packaging; CB: Cardboard packaging; WM: Wholemeal pasta; Fried 1: Fried in a frying pan with margarine; [a] Prior to frying, potato cultivar 1 was boiled; [b] Purchased pre-fried frozen chips.

The influence of cooking on phthalate concentrations in several vegetables is summarised in Table 40. Processing carrots – i.e. boiling or steaming – did not seem to have a real influence, since all concentrations were in line with the concentrations determined in the raw carrot samples. On the other hand, concentrations of DMP, DEHP and to a minor degree also DnOP in boiled or steamed cauliflower were much lower than what was observed in the corresponding unprocessed sample. Furthermore, DEP and BBP were not quantifiable after the cauliflower sample was boiled and steamed, respectively. Onion and paprika were heated in a frying pan with margarine. Almost no differences could be observed before and after frying, except for DEHP in paprika, of which the concentration reduced from 71.77 to 22.06 µg/kg product after frying.

Table 40: Phthalate concentrations (average of two subsamples) in raw and processed vegetables (in µg/kg product).

Food	Type	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Carrot	Raw	ND	0.38	1.06	ND	0.42	13.50	0.19	ND
	Boiled	<0.05	0.21	1.72	ND	0.35	25.66	0.12	ND
	Steamed	ND	ND	<1.00	ND	0.49	15.58	0.11	ND
Cauliflower	Raw	2.37	0.40	1.97	ND	0.42	47.39	<0.10	0.46
	Boiled	0.09	<0.15	1.11	ND	0.27	<7.25	ND	ND
	Steamed	0.13	0.46	1.75	ND	<0.20	18.90	ND	ND
Onion	Raw	ND	0.44	<1.00	ND	0.28	56.71	ND	ND
	Fried 1	ND	0.33	1.26	ND	0.38	35.39	ND	<0.15
Paprika	Raw	ND	0.39	4.82	3.39	0.88	71.77	0.22	0.17
	Fried 1	ND	<0.50	2.63	2.63	0.66	22.06	ND	ND

ND: not detected; <value: detected, but lower than LOQ value; Fried 1: Fried in a frying pan with margarine.

Levels of phthalates in minced meat, pork chop and salmon before and after cooking are shown in Table 41. Frying, in a frying pan with margarine as well as in a non-stick frying pan without margarine, did not really affect the phthalate concentrations determined in meat and fish, since all concentrations remained more or less stable after processing. However, DiBP en DEHP levels in meat or fish fried with margarine (in a frying pan) were always lower compared to the concentrations in the food products fried without margarine (in a non-stick frying pan). Furthermore, grilling augmented the DEHP content in salmon: an increase by a factor of six was observed when no aluminium foil was used and by at least a factor of 27 when salmon was prepared “en papillote” (i.e. wrapped in aluminium foil). Salmon grilled without using aluminium foil also contained less DMP, DiBP and DnBP, but more BBP, than salmon grilled “en papillote”.

Table 41: Phthalate concentrations (average of two subsamples) in raw and processed meat and fish (in µg/kg product).

Food	Type	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Minced meat	Raw	0.95	ND	5.14	4.30	<0.75	66.9	ND	ND
	Fried 1	<1.50	ND	4.56	<4.50	ND	37.9	ND	ND
	Fried 2	<1.00	ND	4.88	4.11	ND	49.3	ND	ND
Pork chop	Raw	ND	0.21	2.38	ND	0.36	285	0.11	<0.15
	Fried 1	ND	0.23	2.68	<2.30	0.62	191	ND	0.30
	Fried 2	ND	0.18	3.77	<2.30	0.72	323	<0.10	0.28
Salmon	Raw	1.60	<1.00	5.80	8.08	1.36	154	ND	ND
	Fried 1	1.63	<1.25	5.21	9.42	1.70	148	ND	ND
	Fried 2	1.37	ND	6.54	8.37	<1.00	262	ND	ND
	Grilled 1	0.98	<0.75	4.04	8.89	1.59	915	ND	ND
	Grilled 2	1.26	<0.75	4.59	9.62	1.38	4,253	ND	ND

ND: not detected; <value: detected, but lower than LOQ value; Fried 1: Fried in a frying pan with margarine; Fried 2: Fried in a non-stick frying pan without margarine; Grilled 1: Grilled in oven without aluminium foil; Grilled 2: Grilled in oven with aluminium foil (“en papillote”).

Besides the effect of cooking at home, also differences in phthalate levels were observed between cultivars, varieties and/or packaging types of a certain food product. Table 39, for example, shows that in this study, potatoes pre-fried at home contained less phthalates than frozen pre-fried

potatoes bought from a shop. Furthermore, the investigated cultivar for potato chips (cultivar 2) contained five times more DEHP than the waxy cultivar (cultivar 1). Although the two considered rice varieties were produced by the same company, DiBP, DnBP, BBP and DEHP contents were higher in loose rice than in the boil-in-bag variety. Finally, white pasta packed in cardboard had higher DiBP, DnBP and DEHP contents than wholemeal pasta (same brand, also packed in cardboard) and white pasta (another brand) packed in plastic.

II.6.4 Discussion

This study investigated the influence of cooking at home on phthalate concentrations in 15 types of foods, including starchy products, vegetables and meat and fish. In the next sections, the effect of cooking as well as differences in cultivars, varieties and packaging types will be discussed. The results obtained in this survey will also be compared with results from other studies, although this was not easy, since only one study concerning phthalates could be found that did similar research.

II.6.4.1 Influence of cooking at home

A decline in phthalate concentration after processing can be influenced by several factors. At first, phthalates can degrade during cooking into phthalic acid with monoester phthalates as intermediates (Cousins et al., 2003; Wolfe et al., 1980). More volatile compounds – i.e. with high vapour pressures such as DMP and DEP – can also evaporate during processing (Cousins et al., 2003). Secondly, phthalates are lipophilic and therefore tend to concentrate in the lipid phase of foodstuffs (Fankhauser-Noti et al., 2006). Since some food products lose fat when they are being processed – e.g. minced meat loses 5% fat during frying (Superior Health Council, 2005) and salmon about 20% (Bayen et al., 2005) – phthalates are removed together with the fat phase. Lastly, phthalate concentrations can be “diluted” because food products take up water during cooking. This was the case for e.g. pasta and rice, of which the weight augmented after boiling (Table 38).

On the other hand, cooking can also result in an increase of phthalate concentrations. For instance, although the rise of DEHP in salmon after grilling was rather unexpected, it was typically caused by a contamination that occurred in the microwave (Table 41). Furthermore, substances like DEP, DiBP, DnBP and DEHP can be present in coatings of non-stick cookware products (Bradley et al., 2007). Thus, these contaminants might have migrated from the coating of the non-stick frying pan into meat and fish during frying without margarine. Some foodstuffs lost weight during cooking, e.g. potatoes (Table 38). Because of this weight loss, phthalate levels after processing are “concentrated”. Lastly, phthalate levels can rise in foods because some food products take up (contaminated) fat during cooking. For instance, onion takes up 10% fat during frying and pork chop about 2% (Superior Health Council, 2005). However, the margarine and vegetable oil that were used for frying and deep-frying, respectively, contained almost no phthalates (results not shown), so this contamination pathway may be excluded.

During each of the investigated cooking processes, a combination of the factors described above influenced the phthalate concentrations in the foods after cooking. Thus, depending on which food item and which cooking process was investigated, cooking will ultimately result in an overall decline or increase of the present phthalate levels.

II.6.4.2 Influence of cultivar, variety and packaging type

Pre-frying potatoes at the factory resulted in higher concentrations for six out of eight investigated compounds in comparison with pre-frying potatoes at home (Table 39). Possible reasons therefore might be that potatoes in the factory come more in contact with materials that can contain phthalates (e.g. conveyor belts) and have for instance an extra packaging and transport step compared to potatoes that are pre-fried at home (Medeiros Vinci et al., 2012). In addition, the oil, in which the potatoes were pre-fried at the factory, might have contained more phthalates compared to the oil that was used in this study. Besides that, differences in phthalate levels might also be due to another (country of) origin of the potatoes, which will probably also be the reason why potato cultivar 2 contained more DEHP than potato cultivar 1.

Differences in DiBP, DnBP and DEHP concentrations were observed between white pasta and wholemeal pasta (Table 39), although both pasta varieties were packed in cardboard and were produced by the same company. The use of other ingredients and processing steps will most likely cause this dissimilarity. Additionally, for the samples analysed in this survey, it could be noticed that foods packed in cardboard contained more DiBP and to a lesser degree also more DnBP, BBP and DEHP than foods packed in plastic (Table 39). DiBP and other phthalates as well are often used in printing inks to improve surface adhesion, flexibility and wrinkle resistance (BfR, 2007; Castle et al., 1989; MAFF UK, 1995; Nerin et al., 1993). It is most likely that the (possibly recycled) cardboard, in which the foods were packed, contained (traces of) phthalate containing printing inks and that DiBP and other substances migrated from the cardboard into the foods during storage.

II.6.4.3 Comparison with other studies

Comparable research to this survey was done by Ishida (1993). This Japanese researcher investigated the occurrence of DEHP in chicken eggs, liver and meat before and after several cooking processes, i.e. freeze-drying, deep-frying in vegetable oil, frying in a frying pan with vegetable oil, grilling and boiling. All cooking methods created a reduction in DEHP concentration in every examined food product, which was – with the exception of DEHP in grilled salmon – more or less also the case in this study. Although the effect of cooking was similar to what was observed in this survey, it should be noticed that DEHP concentrations in the uncooked Japanese food products were much higher than in this study. For instance, raw chicken meat contained 1.27-3.54 mg DEHP per kg product, which is about ten times higher than what was determined in Belgian raw meat (Table 41). Since, to the authors' knowledge, no other researchers did comparable research, further research is needed to confirm or deny these results.

II.6.4.4 Limitations of the study

This survey is – to the authors' knowledge - the first study that investigated the occurrence of eight phthalate compounds (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) in several starchy products, vegetables and (meat and) fish products before and after cooking. However, some limitations have to be discussed. First of all, phthalates are hard to analyse, since they are omnipresent in the (laboratory) environment (David et al., 2003). To comply with the fact that sample contamination can occur in every step of the analytical procedure, the decision was made to analyse every sample in duplicate. Secondly, the analysis of phthalates is time-consuming, which definitely limited the amount of samples that could be measured. In this study, the choice was made to consider at least three food products and three cooking methods for every food category, i.e.

starchy products, vegetables and meat and fish. Due to this limited amount of food products, it might be that the statistical tests that were done do lack some power; an idea about the variance that would be encountered was lacking before setting up the study. However, even with the small sample size considered in this study, low *p*-values were obtained and comparable trends were seen between food categories and between cooking processes. Finally, the effect of cooking on phthalate levels not only seemed to depend on the applied cooking process, but also on the type of food investigated. Therefore, it is desirable that more food samples will be analysed in the future.

II.6.5 Conclusions

This study investigated the occurrence of eight phthalates (DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP and DnOP) in food products using different cooking methods. Before as well as after processing, DEHP was the most abundant phthalate compound, followed by DiBP and BBP. During preparation, several factors were simultaneously responsible for changes in the phthalate concentrations in the examined foodstuffs. Some of these factors influenced phthalate contents in a positive way (i.e. a reduction after heating) and others in a more negative way. However, overall phthalate concentrations mostly declined after cooking at home. The effect of cooking on phthalate levels not only seemed to depend on the applied cooking process, but also on the type of food investigated. For instance, food packed in cardboard contained more DiBP, DnBP, BBP and DEHP than food packed in plastic. Since this study was the first one describing the effect of cooking on eight phthalate levels in starchy products, vegetables and (meat and) fish, further research is definitely desirable.

III. Modelling phthalates in foods and their related exposure in the Belgian adult population

This part – containing two chapters – reports on the results of the work conducted regarding modelling the occurrence of phthalates in foods and their related exposure in the Belgian adult population.

The first chapter describes the modelling framework of "EN-forc": the ENvironmental Food transfer model for ORganic Contaminants. This model comprises both mechanistic and empirical relationships (see Section I.6.2.1) and is developed for the prediction of the occurrence of organic contaminants in a hundred basic agricultural products (fruits, vegetables, grains, meat, milk, eggs, and so on) as a result of environmental transfer (root uptake, deposition, feed intake, and so on). Contaminant concentrations in soil and plants are based on a dynamic model, while levels in groundwater, drink water and animal products are calculated using steady-state equations (see Section I.6.2.1). The modelling framework of EN-forc is illustrated schematically in Figure 11. During this PhD project, the EN-forc model was validated for the prediction of concentration levels of four phthalates in agricultural products, namely DEP, DnBP, BBP and DEHP.

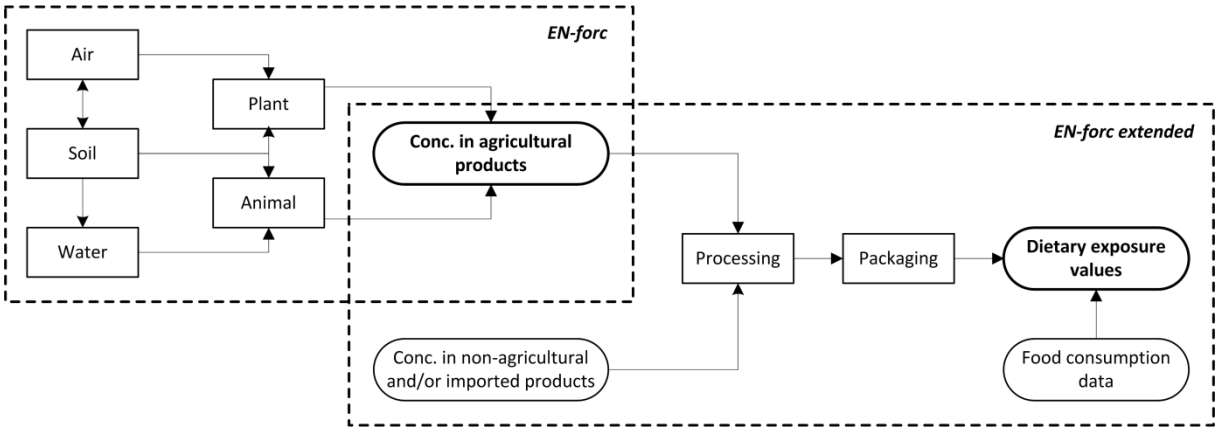

Figure 11: Schematic overview of "EN-forc" and "EN-forc extended".

In the second chapter of this part, extensions to the EN-forc model are described. Besides environmental transfer, "EN-forc extended" also considers additional contamination pathways of DEP, DnBP, BBP and DEHP in foods like processing and packaging. By making use of food recipes, this extended version is able to predict contaminant levels in more than thousand different processed and/or packaged food products (Figure 11). This chapter also reports on the simulations that were done with the extended version of the EN-forc model to predict the dietary exposure of the Belgian adult population to DEP, DnBP, BBP and DEHP using a semi-probabilistic approach (see Section I.6.2.1). For this purpose, the data from the Belgian National Food Consumption survey of 2004 (Devriese et al., 2006) were linked to the model outputs of EN-forc extended (Figure 11).

III.1 EN-forc – Modelling the environmental transfer of phthalates into Belgian agricultural products

Fierens, T., Cornelis, C., Standaert, A., Sioen, I., De Henauw, S. and Van Holderbeke, M. (2014). ***Modelling the environmental transfer of phthalates and polychlorinated dibenzo-p-dioxins and dibenzofurans into agricultural products: the EN-forc model.*** *Environmental Research, 133, 282-293.*

Abstract

This study aimed to predict the occurrence of four phthalates in environmental and agricultural media from observed concentrations in air, sludge, manure and concentrate. For the environmental and agricultural fate modelling, the newly developed multimedia model "EN-forc" (ENvironmental Food transfer model for ORganic Contaminants) was used. To validate EN-forc calculations, the predicted concentrations of the considered chemicals in soil, groundwater, drinking water, plants and animal products were compared with both observed and modelled concentrations available in literature. For the majority of the considered matrices, predicted phthalate levels differed one order of magnitude at most with observed concentrations. Unfortunately, the transfer models implemented in EN-forc lacked power to predict levels of some phthalates in pasture, root crops and/or tubers. Concentrations of phthalates in offal could not be predicted due to the absence of suitable models that have an acceptable level of complexity to implement in EN-forc. For this type of food products, further research is highly encouraged. In a next step, the modelling framework of EN-forc will be extended in order to be able to predict human dietary exposure to organic chemicals like phthalates.

III.1.1 Introduction

Phthalates, or diesters of *ortho*-phthalic acid, are a group of organic chemicals with a wide range of user applications. Among others, they are used in cosmetics, floorings, building materials, clothing, medical devices, food contact materials, inks and adhesives. To date, more than 30 different phthalate compounds are commercially available on the market (Cao, 2010; David et al., 2003; Heudorf et al., 2007; Schettler, 2006). Some of them – as well as their metabolites – are suspected to be endocrine disrupting compounds. For example, diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) are added as Category 1 substances to the European priority list of chemicals with potential endocrine disrupting activities (European Commission, 2014b). Since phthalates are not covalently bound in their user applications, they can relatively easily leak from them during manufacturing, use and after disposal (Heudorf et al., 2007; Schettler, 2006). As a result of their widespread use, phthalates are omnipresent in the environment, in spite of the fact that they are rapidly degraded (Peterson and Staples, 2003). Concentration levels of phthalates have been reported in air (outdoor and indoor), surface water, groundwater, drinking water, soil, sediment, sludge, vegetation, cow's milk, and so on (Bono-Blay et al., 2012; Fierens et al., 2012b; Fromme et al., 2002; Rakkestad et al., 2007; Rudel et al., 2003; Sablayrolles et al., 2005; Santana et al., 2014; Serodio and Nogueira, 2006; Teil et al., 2006; Vikelsoe et al., 2002).

Analysing phthalates in environmental and agricultural media is complex, expensive and time-consuming (David et al., 2003; Fierens et al., 2012c). In order to overcome these difficulties, modelling the environmental fate of these chemicals can offer a relatively inexpensive and rapid

solution. Moreover, modelling can produce results applicable to past, future or hypothetical scenarios, which are impossible to monitor experimentally. This additional advantage is especially interesting for policy makers in the context of guideline development (WHO, 2005).

The occurrence of phthalates in environmental and agricultural media has already been modelled before. For instance, Müller et al. (2003) predicted the environmental fate of DEHP, DnBP, diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) in Denmark using the "European Union System for the Evaluation of Substances" (EUSES) model. Other researchers reporting environmental concentrations calculated with EUSES are, among others, Cousins and Mackay (2001) for DEHP and Effting and van Veen (1998) for BBP. In another publication, Cousins and Mackay (2003) used a regional population based model (RPM) to predict the environmental fate of DnBP and DEHP for regions located in Western Europe or Eastern America. Both EUSES and RPM are steady-state models that use environmental emission data as model inputs.

The studies mentioned above all prove that modelling can be a good alternative for obtaining concentration levels of phthalates in environmental and agricultural media. However, with the purpose of assessing human dietary exposure to phthalates and in order to set up food safety guidelines, the food concentrations calculated by these models are not refined enough. For example, the EUSES model predicts contaminant concentrations in one type of fish, one type of meat and one type of leafy vegetable in order to calculate human exposure to chemicals via the intake of fish, meat and leafy vegetables (Vermeire et al., 2005; 1997).

In this paper, the framework of the newly developed multimedia model "EN-forc", the ENvironmental Food transfer model for ORganic Contaminants, is described. This deterministic model – based on both mechanistic and empirical relationships – predicts concentrations of organic contaminants in soil, groundwater, drinking water, numerous food and feed crops and several animal products (meat, liver, kidney, milk and eggs). Contaminant concentrations in soil and plants are based on a dynamic model, while levels in groundwater, drinking water and animal products are calculated using steady-state equations. Observed concentrations in air (total air, gas phase, particles and/or particle deposition flux), surface water, sludge, manure and other media are used as input data. Thus, all media of interest for the environmental transfer of a certain chemical in the primary food chain can be considered. The EN-forc model is able to run scenarios for background as well as (historically) contaminated areas. Also food crisis situations like the Belgian dioxin crisis of 1999 for instance, can be simulated with the model. This way, EN-forc can be useful to both researchers and policy makers working in the food safety domain. In this paper, the use of the EN-forc model is demonstrated for the prediction of background concentrations of the phthalates DEP, DnBP, BBP and DEHP in Belgian environmental and agricultural media. For this purpose, available data as much as applicable to Belgium are used as input for the model. To validate the EN-forc model, calculated concentrations are compared with both observed and modelled (steady-state) data from literature.

III.1.2 Material and methods

III.1.2.1 Modelling framework

EN-forc is based on two other modelling frameworks developed at our research institute, i.e. XtraFOOD (Seuntjens et al., 2006) and S-Risk (Cornelis et al., 2013). XtraFOOD, which is the acronym for "Xenobiotics TRAnsfer in the primary FOOD chain", is a chain model that analyses the impact of contaminants in primary food products and calculates human dietary exposure to these compounds (Seuntjens et al., 2006). S-Risk on the other hand, is a steady-state, mass-conservation based model for the calculation of exposure and risk of humans to soil and groundwater contaminants (Cornelis et al., 2013).

A schematic overview of the EN-forc model is depicted in Figure 12. EN-forc includes six different modules to describe the environmental system: chemical, soil, water, air, plant and animal. In the following sections, the structure of every module is described. Since most equations have already been described in detail in the reports of XtraFOOD and S-Risk (Cornelis et al., 2013; Seuntjens et al., 2006), only base equations and EN-forc specific adaptations are mentioned. A summary of the base equations can be found in the Annexes. EN-forc is programmed in MATLAB® version R2011b (The MathWorks, Inc.).

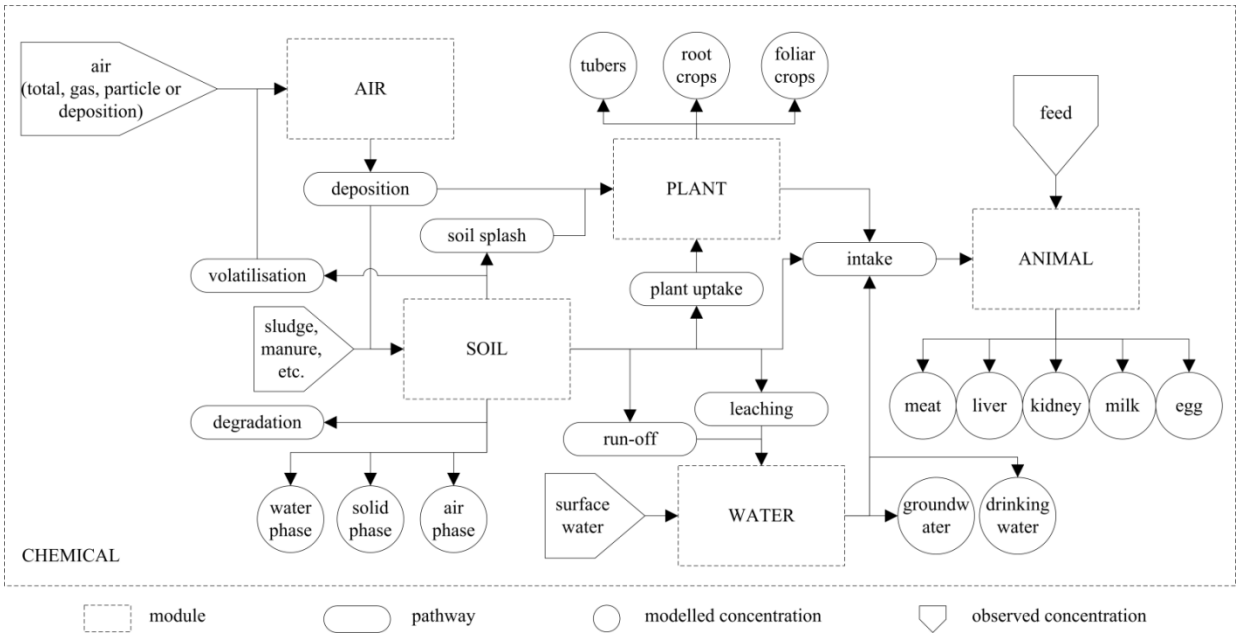

Figure 12: Schematic overview of EN-forc.

a) Chemical module

Various physico-chemical properties are required to predict the environmental fate of a chemical (see Section III.1.2.2). In the "chemical" module of EN-forc, the following physico-chemical properties of a compound are calculated: the organic carbon-water partition coefficient (K_{oc}), the soil-water partition coefficient (K_d), the dimensionless Henry coefficient (H'), the Henry coefficient at temperature T ($H(T)$) and the diffusion coefficient in air (D_a) and water (D_w). To calculate K_{oc} for non-

dissociating organic substances, EN-forc uses the quantitative structure-activity relationships (QSARs) of Sabljic and Gusten (1995). The equation formulated for esters is considered to be appropriate for phthalates.

b) Soil module

Chemical transfer in soil is described using the first-order kinetic model of Vissenberg and van Grinsven (1995). This model calculates changes in soil concentrations (C) during a crop's growing season. Within a single homogeneous soil compartment, the evolution of contaminant concentrations over time can be expressed by a linear first-order differential equation. In this equation, parameter "k" represents the sum of all soil losses, i.e. transport and/or transformation processes. The considered processes for phthalates in EN-forc are: volatilisation (k_v), run-off (k_r), plant uptake (k_p), biodegradation (k_b) and leaching (k_l). To calculate k_p, different equations are used for foliar crops, root crops and tubers (Trapp and Legind, 2011). Contaminants do not only disappear from the soil; they can also be added to the soil. The overall contaminant load to the soil is characterised by parameter "I". Examples of contaminant loads are atmospheric deposition and the use of fertilisers, manure, compost, sludge, irrigation water, pesticides, and so on (Vissenberg and van Grinsven, 1995). For the environmental fate modelling of phthalates, soil loads due to atmospheric deposition (I_A) and due to the use of manure (I_M) and sludge (I_S) are considered. These inputs are modelled as continuous inputs of the soil-plant system throughout the year. In the equation of I_A, parameter "I_v" represents the fraction of particles intercepted by vegetation, which is calculated for every plant according to Baes et al. (1984). Unknown soil properties necessary for these equations – for instance, the volumetric air (ϑ_a) and water (ϑ_w) content – are calculated according to van Genuchten (1980) and Vereecken et al. (1989).

c) Water module

The "water" module of EN-forc predicts contaminant concentrations in groundwater (C_{gw}) and drinking water (C_{wp}). The concentration in groundwater is calculated from the leaching flux and assumes complete mixing in a homogeneous groundwater layer below the soil compartment (OVAM, 2005). If plastic drinking water pipes are situated in a soil layer contaminated with organic chemicals, diffusion through the pipe wall can take place. Concentrations in drinking water due to this process are calculated in EN-forc according to Vonk (1985).

d) Air module

To describe chemical deposition from air on soil and plants, a contaminant particle deposition flux (F_p) and a contaminant concentration in the gas phase ($C_{g,a}$) have to be added to the "air" module of EN-forc. If no values for F_p and/or $C_{g,a}$ are available, they are calculated from an entered total air concentration ($C_{a,a}$) or an observed contaminant concentration in suspended particles ($C_{p,a}$) (Meneses et al., 2002).

e) Plant module

The dynamic plant models of Trapp and Legind (2011) are used to predict uptake, translocation, elimination and bioaccumulation of organic chemicals in plants (C_p). A distinction is made between

foliar crops (e.g. pasture, cabbage, fruit and grain), root crops (beet and carrot) and tubers (potato). In these models, plants are assumed to take up chemicals via the transfer from soil to plant via root uptake and – for foliar crops also – from air via gas phase exchange. In EN-forc, wet and dry particle deposition (C_{dep}) and adherence of splashed soil particles (C_{spl}) are incorporated as additional input pathways of chemicals to foliar crops (Meneses et al., 2002; Samsoe-Petersen et al., 2003). Unlike the first transfer route, these two pathways are calculated statically.

f) Animal module

The "animal" module of EN-forc predicts contaminant concentrations in meat, liver, kidney, milk and/or eggs for several types of animals. First, animal exposure ($J_{f,year}$) is calculated by summing the contaminant intakes (J_x) via the ingestion of pasture, feed, concentrate, soil, water and/or milk. A distinction is made between contaminant intake during summer ($J_{f,summer}$) and winter ($J_{f,winter}$), since diet patterns differ between summer and winter. Chemical transfer to animal products (C_y) is calculated using biotransfer factors (BTFs): concentration in product per unit of daily contaminant intake (day kg^{-1}). When a BTF is not available for a chemical and/or animal product, this parameter is calculated by using relationships reported in literature. For the calculation of BTF values of chemicals in eggs, the equation of McKone (1993) is used. The equations of both Rosenbaum et al. (2009) and Travis and Arms (1988) are implemented in EN-forc to calculate the biotransfer of chemicals to beef meat and cow's milk. These equations are also used for the calculation of BTF values of chemicals in meat from other animals (e.g. pork and chicken) and in offal (liver and kidneys), since no existing models could be found that had an acceptable level of complexity to implement in EN-forc.

III.1.2.2 Input data

EN-forc requires several types of input data: physico-chemical properties, contaminant concentrations, biotransfer factors, environmental characteristics, and so on. In this section, an overview is given of the different required data and the sources used to obtain them. All input data are stored in a SQLite relational database system (http://www.sqlite.org/), allowing flexible querying and combining heterogeneous data.

a) Physico-chemical properties

The physico-chemical properties that were added to EN-forc for the considered compounds are given in Table 42. Properties such as molecular mass (M), water solubility ($S(T)$), vapour pressure ($P(T)$) and octanol-water partition coefficient (K_{ow}) were mainly taken from EU risk assessment reports (ECB, 2004; 2007; 2008) and from the Handbook of Environmental Chemistry for phthalate esters (Staples, 2003). In the third chapter of this handbook, biodegradation studies were reviewed, of which median biodegradation constants in soil (k_b) and water (k_{wb}) were derived to enter into the model (Peterson and Staples, 2003). Volumetric washout factors for particles (W_p) were obtained from Ligocki et al. (1985). Values for the organic carbon-water partition coefficient (K_{oc}) were taken from a report of the Dutch National Institute of Public Health and the Environment (Lijzen et al., 2001). Loss rates due to metabolisation (α_{meta}) and photodegradation (α_{photo}) in plants were set to zero. Regarding the relative bioavailability of phthalates in soil versus feed (RBA_{soil}), the worst-case scenario – i.e. a value of 1.0 – was assumed. Finally, the permeation coefficient of plastic drinking water pipes (D_p) was set to 2E-6 m^2 day^{-1} for all considered phthalates (Lijzen et al., 2001).

Table 42: Physico-chemical properties, biotransfer factors and contaminant concentrations used as inputs to the EN-forc model.

Parameter	Unit	DEP	DnBP	BBP	DEHP	Reference
Physico-chemical properties						
M – molecular mass	g mol^{-1}	222.2	278.4	312.4	390.6	(ECB, 2004; 2007; 2008; Staples, 2003)
S(T) – water solubility	mg L^{-1}	912 @ 25 °C	10 @ 20 °C	2.8 @ 20 °C	0.003 @ 20 °C	(ECB, 2004; 2007; 2008; Staples, 2003)
P(T) – vapour pressure	Pa	2.2E-1 @ 25 °C	9.7E-3 @ 25 °C	1.1E-3 @ 20 °C	3.4E-5 @ 20 °C	(ECB, 2004; 2007; 2008; Staples, 2003)
W_p – volumetric washout factor for particles	-	25000	25000	8000	20367	(Ligocki et al., 1985)
Log K_{ow} – octanol-water partition coefficient	-	2.32	4.57	4.84	7.50	(ECB, 2004; 2007; 2008; Staples, 2003)
Log K_{oc} – organic carbon-water partition coefficient	L kg^{-1}	2.64	2.98	3.91	5.37	(Lijzen et al., 2001)
k_b – soil biodegradation constant	yr^{-1}	138.7	30.1	-	6.9	(Peterson and Staples, 2003)
k_{wb} – water biodegradation constant	yr^{-1}	-	-	147.8	-	(Peterson and Staples, 2003)
Biotransfer factors (BTFs)	[mg kg^{-1}] [mg day^{-1}]$^{-1}$					
BTF – cattle, meat		-	-	-	0.0002	(Müller et al., 2003)
BTF – cattle, milk		-	0.039	-	0.0069	(Blüthgen, 2003; Jarosova, 2006)
BTF – pig, meat		-	0.023	-	0.25	(Jarosova et al., 1999)
BTF – pig, liver		-	0.023	-	0.025	(Jarosova et al., 1999)
BTF – pig, kidney		-	0.007	-	0.025	(Jarosova et al., 1999)
BTF – poultry, meat		-	2.29	-	0.137	(Jarosova et al., 2009)
BTF – poultry, liver		-	0.521	-	0.03	(Jarosova et al., 2009)
Contaminant concentrations						
$C_{bg,gw}$ – background concentration in groundwater	mg m^{-3}	0.10	0.10	0.01	0.05	(Fierens et al., 2012b)
$C_{bg,wp}$ – background concentration in drinking water	mg m^{-3}	0.03	0.03	0.01	0.03	(Fierens et al., 2012c)
$C_{t=0}$ – initial concentration in soil	mg kg^{-1} dm	0	0	0	0	-
$C_{p,t=0}$ – initial concentration in plants	mg kg^{-1} dm	0	0	0	0	-
C_M – contaminant concentration in manure	mg kg^{-1} dm	-	0.08	0.08	0.12	(Vikelsoe et al., 2002)
C_S – contaminant concentration in sludge	mg kg^{-1} dm	30	10	20	100	(Sablayrolles et al., 2005)
F_p – contaminant particle deposition flux	mg m^{-2} yr^{-1}	1.6E-1	3.3E-1	5.4E-2	8.7E-1	(Teil et al., 2006)
$C_{g,a}$ – contaminant concentration in air gas phase	mg m^{-3}	8.7E-6	1.7E-5	4.1E-6	1.2E-5	(Teil et al., 2006)
C_{conc} – contaminant concentration in concentrate	mg kg^{-1} dm	7.7E-4	9.4E-4	1.9E-2	2.0E-2	(Fierens et al., 2012b)

b) Contaminant concentrations

The phthalate concentrations that were used as inputs to EN-forc are summarised in Table 42 as well. Levels of the considered phthalate compounds in groundwater (background; $C_{bg,gw}$), drinking water (background; $C_{bg,wp}$) and concentrate (C_{conc}) were taken from previous Belgian monitoring studies (Fierens et al., 2012b; 2012c). When levels were below the limit of detection, the value of the detection limit was taken. Concentrations in concentrate were converted from "mg kg^{-1} fw" to "mg kg^{-1} dm" by considering a dry matter content of 85%. The French survey of Teil et al. (2006) was used to obtain phthalate particle deposition fluxes (F_p) and concentrations of phthalates in the air gas phase ($C_{g,a}$) or in total air ($C_{a,a}$), since Belgian data were lacking. Initial phthalate concentrations in soil ($C_{t=0}$) and plants ($C_{p,t=0}$) were set to zero. Data from Vikelsoe et al. (2002) and Sablayrolles et al. (2005) were adopted to obtain European phthalate concentrations in manure (C_M) and sludge (C_S), respectively. For manure, a dry matter content of 25 % was assumed for converting to "mg kg^{-1} dm". Since phthalate levels in pasture were overestimated by the EN-forc model (i.e. a difference of two orders of magnitude at most ; see Section III.1.3.2), the decision was made to replace the modelled phthalate concentrations in pasture by Belgian observed levels (Fierens et al., 2012b) to calculate phthalate concentrations in animal products. Assuming a dry matter content of 16%, observed levels of DEP, DnBP, BBP and DEHP in pasture were 2.8E-3; 1.4E-2; 2.2E-3 and 1.1E-1 mg kg^{-1} dm, respectively.

c) Biotransfer factors

As shown in Table 42, measured BTF values were only found for two phthalates, namely DnBP and DEHP. Values for DEHP in cattle were taken from Blüthgen (2003) and Müller et al. (2003); the other BTFs were based on the results of Jarosova and co-workers (1999; 2006; 2009).

d) Environmental characteristics

Soil property values for typical Belgian soil layer types were extracted from the comprehensive database "Aardewerk" (Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993). This database is the digital version of the Belgian National Soil Survey and comprises measurement data on more than 10,000 different Belgian soil profiles, including the fraction of organic carbon (*OC*), sand and clay content, soil thickness (d), soil dry bulk density (ρ_s), *pH-KCl*, and more. For the environmental fate modelling, average property data were calculated for three common Belgian soil types: sand, loam and clay. A further distinction was made between meadow and arable land (Table 43). Based on expert opinion, the soil erosion loss constant (A_s) was set to 2 kg m^{-2} yr^{-1} (dry matter) for loamy arable land and to 0 kg m^{-2} yr^{-1} (dry matter) for the other soil types. The application rate of manure was set to 0.5 kg m^{-2} yr^{-1} (dry matter) (VLM, 2012) and sludge was assumed to be used at a rate of 0.1 kg m^{-2} yr^{-1} (dry matter) on meadows and 0.2 kg m^{-2} yr^{-1} (dry matter) on arable land (European Commission, 2001). The Royal Meteorological Institute of Belgium was consulted with respect to Belgian weather forecast data (RMI, 2013): over the last three decades, the annual temperature (T) in Belgium was about 10 °C (or 283 K) and the annual rainfall (R_n) equalled 0.852 m yr^{-1} on average. The daily drinking water use at a farm was assumed to be 1 m^3 day^{-1}.

Table 43: Properties of three common Belgian soil types: sand, loam and clay. A further distinction is made between meadow and arable land.

Parameter	Unit	Meadow			Arable land			Reference
		Sand	Loam	Clay	Sand	Loam	Clay	
OC – fraction of organic carbon in soil	-	0.0159	0.0163	0.0294	0.0142	0.0108	0.0114	(Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993)
d – soil thickness	m	0.2750	0.2334	0.1989	0.2750	0.2528	0.2048	(Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993)
pH-KCl	-	4.77	5.48	5.40	4.45	5.89	6.24	(Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993)
sand fraction in soil	%	88.28	13.96	35.58	88.02	6.79	33.18	(Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993)
clay fraction in soil	%	2.50	15.37	23.91	2.83	14.73	23.47	(Van Orshoven and Maes, 1988; Van Orshoven and Vandenbroucke, 1993)
A_s – erosion loss	kg dm m^{-2} yr^{-1}	0	0	0	0	2	0	-
Q_M – application rate of manure	kg dm m^{-2} yr^{-1}	0.5	0.5	0.5	0.5	0.5	0.5	(VLM, 2012)
Q_S – application rate of sludge	kg dm m^{-2} yr^{-1}	0.1	0.1	0.1	0.2	0.2	0.2	(European Commission, 2001)

e) Plant and animal related data

Plant properties like dry matter content (*DM*), surface area (*A*) and growing season (t_{growth}) were compiled from various literature sources (Allen et al., 1998; Baes et al., 1984; Dedeene and De Kinder, 2011; Jager and Hamers, 1997; Nubel, 1999; Trapp and Matthies, 1995). For plant yields (*Y*), Belgian and European statistical databases were consulted (Eurostat, 2013; FOD, 2013b). The loss rate due to growth dilution (α_{growth}) of a plant was set to 12.8 yr^{-1} for pasture and to 36.5 yr^{-1} for other foliar crops (Trapp and Matthies, 1995). For tubers and root crops, this loss rate was first set to 36.5 yr^{-1}, but preliminary calculations with EN-forc revealed that this ended in contaminant concentrations that underestimated reality (i.e. differences of about four orders of magnitude compared to observed levels).To overcome these underpredictions, growth rates for tubers and root crops should be modelled in a variable, logistic way like can be done with the newly developed dynamic plant uptake models of Rein et al. (2011), but these models are regrettably too complex to implement into EN-forc. Therefore, in consultation with the developers of the dynamic plant models, it was decided to set the α_{growth} values for tubers and root crops to 0 yr^{-1} in EN-forc.

Animal characteristics were also gathered from several information sources. Most data – for instance, time fractions for summer/winter diet ($t_{f,summer/winter}$) and feed consumption rates (q_x) – were derived from the reports of Jongbloed and Kemme (2005), Kemme et al. (2005), Van Raamsdonk et al. (2007) and from Römkens and co-workers (2007).

III.1.2.3 Scenarios

Phthalates are rapidly degraded in soil (Peterson and Staples, 2003). Consequently, initial concentrations of phthalates in soil were set to zero (see Section III.1.2.2) and model simulations were run until concentration dynamics of the soil-plant system showed a stable pattern over subsequent growing seasons. Phthalate levels in soil and plants were subsequently extracted at the end of the growing season.

III.1.2.4 Validation data

Chemical concentrations predicted by EN-forc were compared with both observed and modelled concentrations, preferably originating from Belgian studies. Phthalate concentrations measured in soil, groundwater, drinking water, pasture, silage, fruits, vegetables, meat, milk, liver, kidney and eggs were mainly taken from previous Belgian studies (Fierens et al., 2012a; 2012b; 2012c) and completed with data from European surveys (Bono-Blay et al., 2012; Jarosova et al., 1999; Santana et al., 2014; Serodio and Nogueira, 2006). Modelled phthalate levels were taken from several European studies, since no data were available for Belgium (Cousins and Mackay, 2001; 2003; Effting and van Veen, 1998; Müller et al., 2003).

III.1.3 Results and Discussion

III.1.3.1 Occurrence in the environment

Calculated concentrations of phthalates in different Belgian soil types are given in Table 44. Although within the same order of magnitude, contaminant levels in soil were generally higher in clay soil than in loamy and sandy soil. Besides, soils from meadows were predicted to be less contaminated with phthalates than soils from arable land. This was mainly due to the higher frequency of sludge applications on arable land (Table 43), which automatically resulted in a higher contaminant load. On

average, DEHP was present in the soil's solid phase for more than 99.9% while percentages of DEP, DnBP and BBP amounted 89.4%, 95.0% and 99.4%, respectively. Average distribution percentages for DEP, DnBP and BBP in the water phase were 10.6%, 5.0% and 0.6%, respectively. Distribution percentages of DEHP in water as well as of all four phthalate compounds in air were less than 0.1% on average. Among the considered phthalate compounds, BBP and DEHP were the most abundant in soil (Table 44). As can be noticed from Table 44, EN-forc predicted soil levels of phthalates that differ one order of magnitude at most with soil levels obtained in Belgian monitoring programs (Fierens et al., 2012b) and European modelling studies (Cousins and Mackay, 2001; Cousins and Mackay, 2003; Effting and van Veen, 1998; 2002; Müller et al., 2003). This was with the exception of DnBP and BBP, of which predicted soil concentrations were two orders of magnitude lower and higher, respectively, compared to literature. A possible reason for this under- and overestimation, respectively, might be the use of too high/low input data (i.e. soil loss rates and soil contaminant loads) in the soil module of EN-forc. For example, of all considered soil losses (see Section III.1.2.1), biodegradation (k_b) and plant uptake (k_p) were the most important loss factors for DnBP and BBP in soil. As can be noticed from Table 42, a soil biodegradation constant for BBP was lacking. So, this parameter had to be estimated from the biodegradation constant in water (k_{wb}). This may have caused an underprediction of the k_b value of BBP in the soil differential equation and thus may have led to an overestimation of the BBP concentration in soil.

Phthalate levels predicted in Belgian groundwater and drinking water are also shown in Table 44. DEHP levels in drinking water were higher than in groundwater whereas the opposite was observed for the remaining phthalates. This dissimilarity might be explained by the fact that plastic drinking water pipes can contain phthalates (especially DEHP) and thus, that drinking water can be additionally contaminated with DEHP as a result of diffusion through the pipe wall (Cao, 2010). Soil type (sand, loam or clay) or land use (meadow or arable land) did not affect the concentrations of phthalates in water (Table 44). EN-forc calculations of phthalates in groundwater and drinking water were generally within the same range as concentrations observed in European monitoring studies (Bono-Blay et al., 2012; Santana et al., 2014; Serodio and Nogueira, 2006). European modelling studies were only available for the phthalates DnBP, BBP and DEHP (Cousins and Mackay, 2003; Effting and van Veen, 1998; Müller et al., 2003). In these studies, modelled concentrations of BBP in drinking water were two orders of magnitude lower than the values predicted by EN-forc and the values observed in European monitoring studies (Bono-Blay et al., 2012; Serodio and Nogueira, 2006). On the other hand, concentrations of DnBP and DEHP were more or less comparable with EN-forc calculations and thus also with monitoring results (Bono-Blay et al., 2012; Santana et al., 2014; Serodio and Nogueira, 2006).

Table 44: Contaminant concentrations in soil (in mg kg^{-1} dm), groundwater (in mg m^{-3}) and drinking water (in mg m^{-3}) calculated by EN-forc for several Belgian soil and land use types and corresponding average (minimum - maximum) levels from monitoring and modelling studies cited in literature.

		Meadow			Arable land			Literature	
Compound	**Medium**	**Sand**	**Loam**	**Clay**	**Sand**	**Loam**	**Clay**	**Monitoring studies**	**Modelling studies**
DEP	Soil	1.1E-5	1.3E-5	2.3E-5	6.3E-5	6.4E-5	8.0E-5	<3.6E-4 (<3.6E-4 - <3.6E-4)[a]	-
	Groundwater	4.3E-2	4.3E-2	4.3E-2	4.8E-2	5.0E-2	5.1E-2	<3.3E-1 (<3.3E-1 – 1.1E+0)[b]	-
	Drinking water	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	1.9E-1[c]	-
DnBP	Soil	3.4E-5	4.1E-5	6.7E-5	1.3E-4	1.3E-4	1.7E-4	3.1E-3 (2.3E-3 – 3.8E-3)[a]	2.9E-3 (1.9E-4 - 4.4E-3)[d,e]
	Groundwater	4.3E-2	4.4E-2	4.3E-2	4.8E-2	4.9E-2	5.1E-2	<2.3E-1 (<2.3E-1 - <2.3E-1)[b]	3.5E-2[e]
	Drinking water	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	<1.0E-2 (<1.0E-2 – 5.2E-2)[c,f]	5.7E-1[e]
BBP	Soil	3.2E-3	3.2E-3	8.0E-3	2.5E-2	1.3E-2	1.7E-2	6.0E-4 (5.6E-4 – 6.4E-4)[a]	-
	Groundwater	1.9E-2	1.8E-2	2.4E-2	1.3E-1	8.7E-2	1.1E-1	<1.9E-1 (<1.9E-1 - <1.9E-1)[b]	-
	Drinking water	1.0E-2	1.0E-2	1.0E-2	1.1E-2	1.1E-2	1.1E-2	3.0E-2[c]	5.8E-4[g]
DEHP	Soil	3.7E-3	4.4E-3	5.2E-3	7.2E-3	7.9E-3	9.8E-3	1.2E-2 (6.4E-3 - 1.7E-2)[a]	8.1E-2 (8.0E-3 – 3.5E-1)[d,e,h]
	Groundwater	2.2E-2	2.2E-2	2.1E-2	2.2E-2	2.3E-2	2.3E-2	<4.6E-1 (<4.6E-1 - <4.6E-1)[b]	2.4E-3[e]
	Drinking water	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	3.0E-2	7.3E-2 (1.0E-2 – 1.9E-1)[c,f]	5.7E-2[e]

[a] Fierens et al. (2012b); [b] Bono-Blay et al. (2012); [c] Serodio and Nogueira (2006); [d] Cousins and Mackay (2003); [e] Müller et al. (2003). Regarding groundwater, concentrations in soil pore water from this publication were used for comparison; [f] Santana et al. (2014); [g] Effting and van Veen (1998); [h] Cousins and Mackay (2001).

III.1.3.2 Environmental transfer to plants

Predicted concentrations of phthalates in foliar crops, root crops and tubers are summarised in Figure 13. Foliar crops were predicted to contain more phthalates than root crops and tubers. This is because foliar crops are – in comparison with root crops and tubers – additionally contaminated with phthalates due to the transfer from wet and dry particle deposition, from air gas phase exchange and from splashed soil particles (see Section III.1.2.1). For all considered phthalates, the contribution of the transfer from splashed soil particles to the total contaminant concentration in foliar crops was negligible (0-0.2%) compared to the transfer from deposition (4.5-30.7%) and the combination of root uptake and gas phase exchange (69.3-95.5%). Within the group of foliar crops, DEHP was the most abundant phthalate compound, followed by DnBP (Figure 13). In root crops and tubers, BBP concentrations were the highest of the considered phthalates.

To validate the concentration levels predicted by EN-forc, phthalate concentration data in food from Belgian monitoring studies as well as from European modelling surveys were taken for comparison (see Section III.1.2.4). Phthalate concentrations in fruits and vegetables predicted by EN-forc were within the same order of magnitude as levels obtained in two earlier monitoring studies (Fierens et al., 2012a; 2012c). An exception to this were the levels of DEP, DnBP and DEHP in root crops and potatoes where EN-forc concentrations differed two to three orders of magnitude compared to observed concentrations (Figure 13). In another former study, phthalate levels in pasture and animal feed (i.e. silage) were determined (Fierens et al., 2012b). Measured levels of DEP, DnBP, BBP and DEHP in silage were comparable to the levels calculated by EN-forc. In pasture, predicted levels were higher than observed ones: a maximal difference of two orders of magnitude was noticed (Figure 13). Plant levels for DnBP, BBP and DEHP were predicted using EUSES by Effting and van Veen (1998) and by Müller et al. (2003). Compared to EN-forc predictions and compared to observed plant levels, EUSES calculations (Effting and van Veen, 1998; Müller et al., 2003) of DEHP in foliar crops seemed to underestimate reality while predictions of BBP in root crops were more than two orders of magnitude higher (Figure 13).

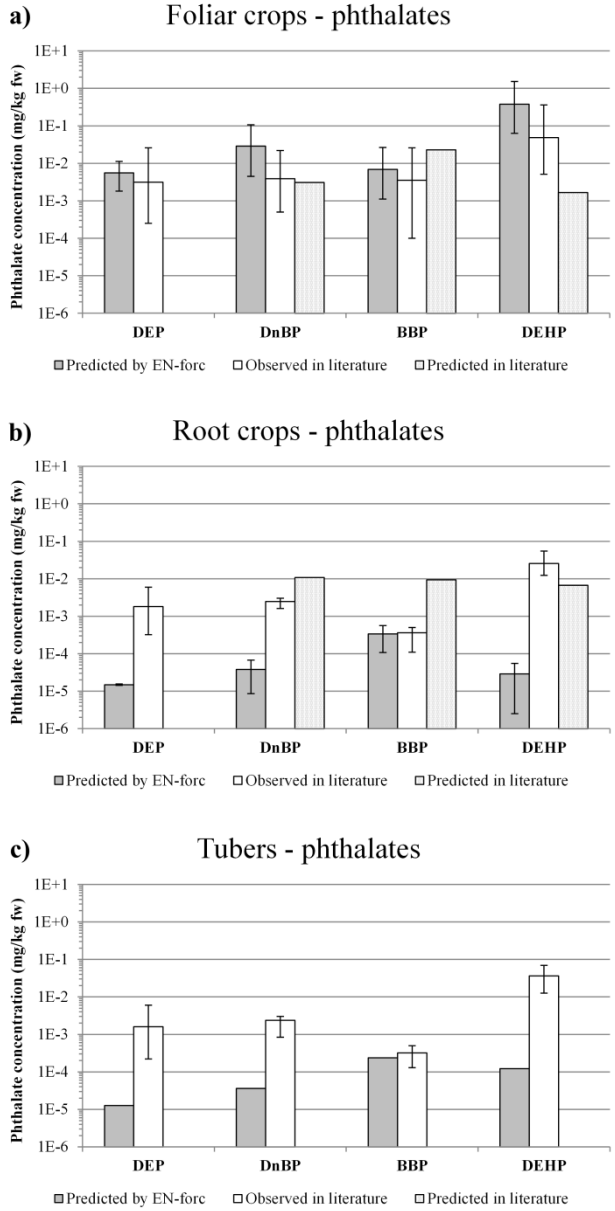

Figure 13: Average concentrations of phthalates in foliar crops (a), root crops (b) and tubers (c) predicted by EN-forc and extracted from monitoring and modelling studies from literature (in mg kg^{-1} fw). For the EN-forc calculations, "foliar crops" included pasture, feed, cabbage, lettuce, fruit, grain, legumes and onion; "root crops" included beet and carrot and "tubers" included potato. The vertical error bars show minimum and maximum contaminant concentrations.

In conclusion, phthalate concentrations in foliar crops predicted by EN-forc were generally in line with measured concentrations observed in literature. On the other hand, levels of DEP, DnBP and DEHP in root crops and/or tubers were underestimated by the dynamic plant models of Trapp and Legind (2011) that were implemented in EN-forc. Even after setting growth loss rate values (α_{growth}) for these plant types to zero (see Section III.1.2.2), EN-forc concentrations were still too low compared to observed levels (Figure 13). Based on these observations, one might conclude that the current root and potato models of Trapp and Legind (2011) are less suited to predict the environmental transfer of some phthalates to root and tuberous vegetables given the available input

data. The older approach of Müller et al. (2003) seems to provide better predictions, since DnBP and DEHP concentrations in root crops predicted by EUSES approximate the measurements quite well (Figure 13). The fact that phthalate uptake in root crops and tubers was underestimated by the plant module of EN-forc, may also be due to the fact that processes like air gas phase exchange, wet and dry particle deposition and adherence of splashed soil particles were not considered in these plant types (Trapp and Schwartz, 2000). Mikes et al. (2009) for instance, revealed that polychlorinated biphenyls and organochlorine pesticides also enter radishes via air gas phase exchange, which might also be true for phthalates. Nevertheless, apart from the limitations of the model structures as such, it has also to be kept in mind that every model has its own application range: using a model outside its range may lead to wrong estimations and thus to wrong conclusions (Trapp and Schwartz, 2000). For instance, the relationship used to predict the transpiration stream concentration factor of a chemical in plants (see equation 10 in Annexes) is based on measurements from chemicals with log K_{ow} values between -0.77 and 5 (Dettenmaier et al., 2009), which means that this relationship is not proven to be valid for DEHP. Another possible factor that can influence the concentrations of phthalates in root crops and tubers is the quality of the model input data. For example, the concentration of DnBP in soil was about two orders of magnitude lower predicted than in reality (Table 44) most likely owing to the use of soil loss rates and soil contaminant loads that were too high and low, respectively (see Section III.1.3.1). This might have directly resulted in the underestimation of DnBP in root crops and tubers. Given all this, it can be concluded that further research regarding modelling the transfer of organic contaminants in root crops and tubers is strongly encouraged.

III.1.3.3 Environmental transfer to animal products

Phthalate concentrations in animal products (eggs, milk, meat and offal) were calculated by multiplying yearly contaminant intakes with corresponding BTF values. Depending on the nature of the BTF values, i.e. derived from literature or modelled (see Section III.1.2.1), different contaminant concentrations were obtained (Table 45).

Eggs from free-range chickens were more contaminated with phthalates than eggs from non-free-range chickens (Table 45). Reason for this is that EN-forc assumes that free-range chickens are – compared to non-free-range chickens – additionally exposed to phthalates through the intake of soil and pasture. The phthalate concentrations calculated with EN-forc for both egg types were in line with real concentrations (Fierens et al., 2012c).

To predict chemical transfer to milk, both the models of Rosenbaum et al. (2009) and Travis and Arms (1988) are implemented in EN-forc for the calculation of BTF values (see Section III.1.2.1). Comparing contaminant levels based on these approaches in cow's milk (Table 45) with levels calculated using observed BTFs revealed that the approach of Rosenbaum was the best method to calculated BTF values for phthalates in cow's milk. Consequently, contaminant concentrations in milk that were based on BTF values from literature or modelled according to Rosenbaum et al. (2009), also agreed better with measured concentrations for phthalates (Fierens et al., 2012b). Modelled contaminant concentrations in cow's milk have also been published for DnBP, BBP and DEHP (Effting and van Veen, 1998; Müller et al., 2003). In general, these modelled concentrations were within the same order of magnitude in comparison with EN-forc results and with observed concentrations.

Table 45: Phthalate concentrations in animal products predicted by EN-forc. For comparison, average (minimum – maximum) contaminant concentrations extracted from literature are also added. All results are expressed in mg/kg fw.

Animal product	Concentration based on	DEP	DnBP	BBP	DEHP
Egg					
EN-forc – chicken (free-range)[a]	BTF from literature	-	-	-	-
	Modelled BTF (McK)	2.2E-7	7.0E-5	1.7E-3	3.0E-2
EN-forc – chicken (non-free-range)	BTF from literature	-	-	-	-
	Modelled BTF (McK)	1.7E-7	3.7E-5	1.3E-3	2.0E-2
Literature – chicken (super market)	Monitoring	<4.0E-3 (<4.0E-3 - <4.0E-3)[b]	<1.0E-3 (<1.0E-3 - <1.0E-3)[b]	<1.0E-3 (<1.0E-3 - <1.0E-3)[b]	<8.0E-3 (<8.0E-3 - <8.0E-3)[b]
Milk					
EN-forc – cow's milk[a]	BTF from literature	-	7.4E-3	-	1.1E-2
	Modelled BTF (Ros)	3.1E-5	9.5E-4	4.1E-4	1.3E-2
	Modelled BTF (Tra)	3.5E-7	5.7E-5	3.6E-5	4.1E-2
Literature – cow's milk	Monitoring	<8.1E-4 (<8.1E-4 - <8.1E-4)[c]	6.3E-4 (<6.1E-4 – 1.0E-3)[c]	5.2E-4 (<4.0E-4 – 1.5E-3)[c]	9.4E-3 (<2.4E-3 – 3.2E-2)[c]
	Modelling	-	7.2E-5[d]	2.2E-4[e]	7.2E-6[d]
Meat					
EN-forc – beef[a]	BTF from literature	-	-	-	3.3E-4
	Modelled BTF (Ros)	5.5E-5	1.9E-3	8.2E-4	2.6E-2
	Modelled BTF (Tra)	3.3E-7	1.9E-4	1.3E-4	4.2E-1
Literature – beef	Monitoring	<5.0E-4[b]	<5.0E-4[b]	<1.0E-4[b]	2.4E-2[b]
	Modelling	-	2.3E-4[d]	1.5E-3[e]	2.3E-5[d]
EN-forc – pork	BTF from literature	-	1.1E-3	-	1.4E-1
	Modelled BTF (Ros)	7.6E-5	2.9E-3	2.5E-3	2.1E-1
	Modelled BTF (Tra)	7.6E-8	4.8E-5	5.7E-5	1.5E-1
Literature – pork	Monitoring	1.4E-3 (2.2E-4 – 5.0E-3)[b,f]	4.2E-3 (5.0E-4 – 1.4E-2)[b,f]	3.5E-3 (1.0E-4 – 1.0E-2)[b,f]	2.6E-1 (1.0E-2 – 4.3E-1)[b,f]
EN-forc – chicken (free-range)[a]	BTF from literature	-	5.4E-4	-	5.1E-4
	Modelled BTF (Ros)	3.3E-4	1.0E-2	1.6E-1	4.4E-1
	Modelled BTF (Tra)	6.6E-10	2.4E-7	5.8E-6	9.5E-4
EN-forc – chicken (non-free-range)	BTF from literature	-	3.1E-4	-	3.8E-4
	Modelled BTF (Ros)	2.7E-4	6.0E-3	1.4E-1	3.2E-1
	Modelled BTF (Tra)	5.5E-10	1.4E-7	5.1E-6	7.0E-4
Literature – chicken	Monitoring	1.9E-3 (2.0E-4 – 5.0E-3)[b]	1.9E-2 (5.0E-4 – 5.5E-2)[b]	8.7E-4 (1.0E-4 – 2.0E-3)[b]	4.1E-2 (1.0E-2 – 8.8E-2)[b]

Animal product	Concentration based on	DEP	DnBP	BBP	DEHP
Liver					
EN-forc – chicken (free-range)[a]	BTF from literature	-	1.2E-4	-	1.1E-4
	Modelled BTF (Ros)	3.3E-4	1.0E-2	1.6E-1	4.4E-1
	Modelled BTF (Tra)	6.6E-10	2.4E-7	5.8E-6	9.5E-4
EN-forc – chicken (non-free-range)	BTF from literature	-	6.7E-5	-	7.8E-5
	Modelled BTF (Ros)	2.9E-4	6.6E-3	1.7E-1	1.8E+0
	Modelled BTF (Tra)	5.2E-10	1.3E-7	4.8E-6	6.6E-4
Literature – chicken	Monitoring	-	4.0E-2 (2.0E-2 – 7.0E-2)[g]	-	2.0E-2 (2.0E-2 - 2.0E-2)[g]
EN-forc – pig	BTF from literature	-	1.1E-3	-	1.4E-2
	Modelled BTF (Ros)	7.6E-5	2.9E-3	2.5E-3	2.1E-1
	Modelled BTF (Tra)	7.6E-8	4.8E-5	5.7E-5	1.5E-1
Literature – pig	Monitoring	-	3.5E-2 (2.0E-2 – 5.0E-2)[g]	-	<1.0E-2 (<1.0E-2 - <1.0E-2)[g]
Kidney					
EN-forc – pig	BTF from literature	-	3.3E-4	-	1.4E-2
	Modelled BTF (Ros)	7.6E-5	2.9E-3	2.5E-3	2.1E-1
	Modelled BTF (Tra)	7.6E-8	4.8E-5	5.7E-5	1.5E-1
Literature – pig	Monitoring	-	<1.0E-2 (<1.0E-2 - <1.0E-2)[g]	-	<1.0E-2 (<1.0E-2 - <1.0E-2)[g]

McK: McKone (1993); Ros: Rosenbaum et al. (2009); Tra: Travis and Arms (1988); [a] To calculate these concentrations, observed levels of phthalates in pasture from Fierens et al. (2012b) were used instead of levels predicted by EN-forc; [b] Fierens et al. (2012c); [c] Fierens et al. (2012b); [d] Müller et al. (2003); [e] Effting and van Veen (1998); [f] Fierens et al. (2012a); [g] Jarosova et al. (1999).

Although intended to calculate the biotransfer of contaminants to beef, the equations of Travis and Arms (1988) and Rosenbaum et al. (2009) are also used in EN-forc to calculate BTF values for pig and chicken meat (see Section III.1.2.1). With the exception of BBP in chicken, phthalate concentrations in beef, pork and chicken modelled by using "Rosenbaum" BTFs corresponded very well with levels from Belgian monitoring studies (Fierens et al., 2012a; 2012c). In comparison with other modelling studies (Effting and van Veen, 1998; Müller et al., 2003), EN-forc predicts similar levels of DnBP and BBP and better concentrations of DEHP in beef (Table 45).

Models calculating BTF values for contaminants in liver and kidney could not be found to implement in the EN-forc model. Therefore, again the "Rosenbaum" and "Travis and Arms" BTF values of phthalates for meat were used (see Section III.1.2.1). As can be noticed from Table 45, observed concentrations were only found in literature for DnBP and DEHP (Jarosova et al., 1999). Comparing these observed data with the EN-forc predictions revealed that neither the Rosenbaum approach (2009) nor the Travis and Arms (1988) method seemed adequate to calculate the biotransfer of phthalates to offal.

III.1.3.4 Relevance of the model

In this study, the occurrence of four phthalates (DEP, DnBP, BBP and DEHP) in several Belgian environmental and agricultural media was predicted by using the EN-forc model. For the majority of the considered media, predicted contaminant concentrations were in line with concentrations observed in monitoring studies. Thus, for chemicals that are hard to analyse or for which chemical analyses are expensive or time-consuming, this study demonstrated that modelling can offer an inexpensive and rapid solution. Of course, this only holds true when the model is first validated for chemicals with a similar chemical and biological behaviour.

In comparison with other existing models, EN-forc calculates chemical concentrations in various types of food products. For instance, compared to the EUSES model, EN-forc considers several types of leafy vegetables (onion, grain, lettuces, etc.) and meat (beef, pork, etc.) while EUSES considers one type of leafy vegetable and meat. In order to predict human dietary exposure to organic chemicals, the EN-forc model calculates contaminant concentrations in food that are more refined.

EN-forc can also be used to predict concentrations of other organic chemicals in environmental and agricultural media, at least, when enough high quality data are available to specify the model inputs. For instance, the environmental transfer of the phthalates DiDP and DiNP – two high-production volume phthalates – could not be modelled by EN-forc due to a lack of sufficient and reliable physico-chemical property data and Belgian background concentrations. In this study, levels of e.g. DnBP and BBP in soil were under- and overestimated, respectively, most likely also as a result of the use of inadequate input data.

The validation of the EN-forc model also revealed that transfer modelling to plants and offal needs to be further investigated. Thus, in the meantime, phthalate concentrations in these kind of products are preferably originating from monitoring instead of from modelling surveys in order to avoid under- or overestimations in dietary intake assessments (Trapp and Schwartz, 2000).

In a next step, the modelling framework of EN-forc will be extended. Potential contaminant pathways like the processing or packing of agricultural food products will be implemented. Import of

food products and the possibility of entering contaminant concentrations in agricultural foods will also be considered in a next version of the EN-forc model. That way, the EN-forc model will be able to predict human dietary exposure to organic chemicals.

III.1.4 Conclusions

This study calculated the environmental transfer of four phthalates (DEP, DnBP, BBP and DEHP) into agricultural products using the newly developed multimedia model "EN-forc". Predicted contaminant concentrations within one order of magnitude compared to observed concentrations were obtained for the majority of the considered media (soil, foliar crops, eggs, milk, beef, etc.). Contaminant concentrations in soils with varying composition (sand, loam or clay) or land use (meadow or arable land) were all within the same order of magnitude. The occurrence of some phthalates in root crops and/or tubers seemed to be underestimated by the models implemented in EN-forc. Furthermore, models with an acceptable level of complexity to implement in EN-forc for the prediction of phthalates in offal were not available in literature. This opens ample opportunity for further research into the prediction of the environmental dynamics of organic chemicals.

Supporting information

A summary of the base equations used in the different EN-forc modules can be found in the Annexes.

III.2 EN-forc extended – Modelling the dietary exposure to phthalates in the Belgian adult population

Fierens, T., Standaert, A., Cornelis, C., Sioen, I., De Henauw, S., Willems, H., Bellemans, M., De Maeyer, M. and Van Holderbeke, M. (2014). ***A semi-probabilistic modelling approach for the estimation of dietary exposure to phthalates in the Belgian adult population.*** *Environment International,73*, 117-127.

Abstract

In this study, a semi-probabilistic modelling approach was applied for the estimation of the long-term human dietary exposure to phthalates – one of world's most used families of plasticisers. Four phthalate compounds were considered: diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP). Intake estimates were calculated for the Belgian adult population and several subgroups of this population for two considered scenarios using an extended version of the EN-forc model. The highest intake rates were found for DEHP, followed by DnBP, BBP and DEP. In the Belgian adult population, men and young adults generally had the highest dietary phthalate intake estimates. Nevertheless, predicted dietary intake rates for all four investigated phthalates were far below the corresponding tolerable daily intake (TDI) values (i.e. P99 intake values were 6.4% of the TDI at most), which is reassuring because adults are also exposed to these phthalates via other contamination pathways (e.g. dust ingestion and inhalation). The food groups contributing most to the dietary exposure were grains and grain-based products for DEP, milk and dairy products for DnBP, meat and meat products or grains and grain-based products (depending on the scenario) for BBP and meat and meat products for DEHP. Comparison of the predicted intake results based on modelled phthalate concentrations in food products with intake estimates from other surveys (mostly based on measured concentrations) showed that the extended version of the EN-forc model is a suitable semi-probabilistic tool for the estimation and evaluation of the long-term dietary intake of phthalates in humans.

III.2.1 Introduction

Phthalates (dialkyl or alkyl aryl esters of *orhto*-phthalic acid) are one of world's most used families of plasticisers. Although mainly used to soften plastic polymers, such as polyvinyl chloride, some phthalates – especially those with a short alkyl chain length – can also be present in printing inks, lacquers, solvents, personal care products, pharmaceuticals and so on (Cao, 2010; Wormuth et al., 2006). Phthalates and their metabolites have been reported to cause detrimental effects on human health. For example, exposure to benzylbutyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP) is associated with increased incidences of asthma and allergies in children (Bornehag et al., 2004; Jaakkola and Knight, 2008). Phthalates are also suspected to disrupt the human endocrine system. Diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), BBP and DEHP are even marked on the European priority list of chemicals with potential endocrine disrupting activities as Category 1 substances, meaning that evidence has been found of endocrine disrupting activity in at least one animal species, using intact animals (European Commission, 2014b).

Human exposure to phthalates can occur via ingestion, inhalation, medical intravenous interventions or via dermal contact (Schettler, 2006). For most phthalates, especially DEHP, food ingestion is the most important exposure pathway (Clark et al., 2011; Fromme et al., 2007b; Schettler, 2006; Wormuth et al., 2006). With respect to Belgium, dietary exposure to phthalates has already been

studied in the Belgian PHTAL project for preschool children and adults (Sioen et al., 2012). In this project, exposure was calculated probabilistically by combining consumption rates of two Belgian food consumption surveys with ranges of phthalate levels measured in 572 different food products sold on the Belgian market (Fierens et al., 2012a; 2012c). Also the effect of home-cooking was considered in this dietary intake study. Analysing all the food items was laborious work, due to the complex methodology of sample handling and analysis in order to avoid external contamination (Fierens et al., 2012a; 2012c). Bearing this in mind, the objective of the current study is to investigate whether modelled phthalate levels in food products could be used instead for the assessment of dietary exposure to phthalates in the Belgian population.

To adequately model phthalate levels in food products, all possible contamination pathways should be considered. Phthalates may enter the food chain as a result of environmental transfer as well as via the migration from contact materials used during cultivation, production, storage or even during cooking at home (Cao, 2010; Dickson-Spillmann et al., 2009; Fierens et al., 2012a). With respect to environmental transfer modelling, various models have been developed and demonstrated for phthalates in the past (e.g., Cousins and Mackay, 2003; Fierens et al., 2014; Müller et al., 2003). There are also existing various models that are able to predict the migration of phthalates from food contact materials into foods or food simulants (Oldring et al., 2014; Poças et al., 2008; 2010). However, to the authors' knowledge, there are currently no models available that consider both pathways simultaneously for the prediction of contaminant concentration levels in food products. Another thing that should be considered during modelling is the effect of processing (e.g. home-cooking) on phthalate levels in food products, since differences in phthalate intake values were noticed between intake assessment scenarios that took into account food preparation and those in which this was not considered (Sioen et al., 2012). A few years ago, a mechanistic model has already been developed for the prediction of bacterial cross-contamination during food preparation in the domestic kitchen (Mylius et al., 2007), but – to the authors' knowledge – a similar type of model is currently lacking for chemical food contaminants like phthalates.

To realise the objective of this study (i.e. to estimate human dietary exposure to phthalates by using modelled concentrations in foods), the ENvironmental Food transfer model for ORganic Contaminants (EN-forc) is used to obtain predicted levels of phthalates in Belgian food products. EN-forc is a model that predicts the occurrence of organic contaminants in environmental and agricultural media starting from observed concentrations in air, sludge, manure and concentrate and from physico-chemical property data (Fierens et al., 2014). Since the EN-forc model only predicts phthalate levels in agricultural products and only as a result of environmental transfer, the modelling framework of the EN-forc model is extended by including the effect of packaging, processing and import on phthalate concentrations in foods. As a case study, the long-term dietary intake of four phthalates – DEP, DnBP, BBP and DEHP – in the Belgian adult population is estimated using a semi-probabilistic modelling approach. In addition to the predicted intake distributions of the studied phthalates, the intake estimates are evaluated against exposure limit values and the contribution of 20 different food groups to the total dietary intake of the four considered phthalates is characterised.

III.2.2 Material and methods

III.2.2.1 Framework for linking data

The framework for linking concentration and consumption data to estimate and evaluate human dietary exposure to organic contaminants with the extended version of the EN-forc model is depicted in Figure 14. The environmental transfer part of the EN-forc model predicts concentrations of organic contaminants in environmental and agricultural media starting from observed concentrations in air, sludge, manure and concentrate and from physico-chemical property data. The modelling framework of this part has been recently described in detail by Fierens et al. (2014) and will therefore not be repeated here. The extensions to the EN-forc model are explained in the following paragraphs. The EN-forc model is implemented in MATLAB® R2011b (The MathWorks, Inc., Natick, Massachusetts, USA).

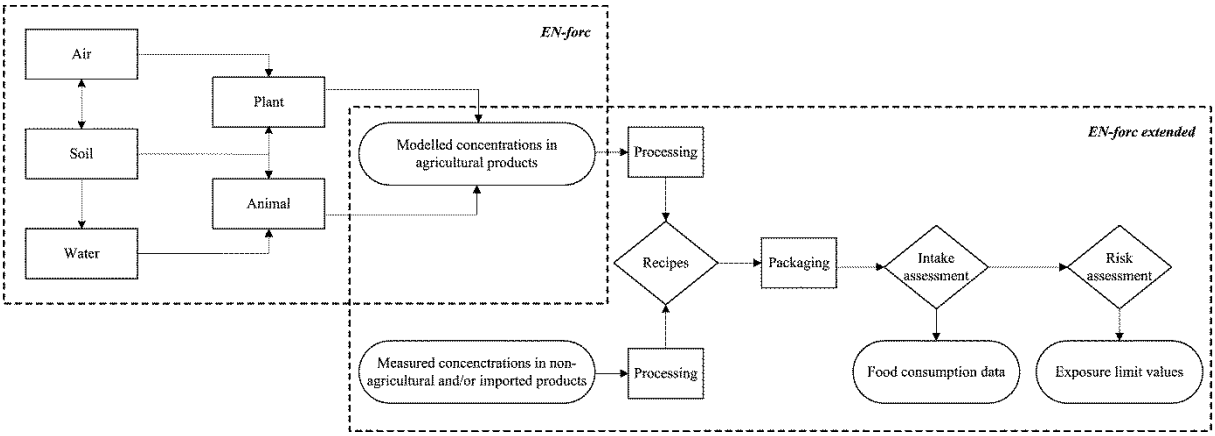

Figure 14: Framework for linking concentration and consumption data to estimate and evaluate human dietary exposure to organic contaminants with the extended version of the EN-forc model.

a) Food recipes

In EN-forc, food products present on the European market are formulated as food recipes, describing their composition of one or more basic agricultural, non-agricultural and/or imported food products. These recipes are all mapped to “FoodEx2” codes. The FoodEx2 coding system was developed by the European Food Safety Authority (EFSA) as a standardised food classification and description system within the European Union (2011a; EFSA, 2011b). EN-forc considers recipes for 1,908 different FoodEx2 codes in total, covering 20 food groups to represent the total European food market (see Table 46 for a full listing). Food recipes were gathered from various sources (cooking books, internet sources, nutrient tables and expertise of dieticians). Food recipes range from very simple, as some products are only composed of one ingredient (e.g. butter is for 100% derived from cow’s milk); others are more complex like white wheat bread, which is considered to contain 60% wheat, 36% tap water, 3% yeast and 1% fat.

Lipophilic chemicals, such as phthalates, tend to concentrate in the fat matrix of a food product. For this reason, EN-forc implements fat correction factors, i.e. the ratio of the fat content of the food product and the fat content of the product's ingredient. For example, butter has a fat correction factor of 19.8 (80% fat in butter with respect to 4.04% in raw cow's milk).

Depending on the type of recipe, processing factors are taken into account for every ingredient and contaminant, since processing (e.g. washing, peeling and boiling) can alter contaminant concentrations in foods (Bayen et al., 2005; Chavarri et al., 2005; Perelló et al., 2008; 2009; Roberts et al., 2008). The EN-forc model considers 13 processing types: unprocessed, washed, peeled, cooked (not specific), baked, boiled, roasted, deep fried, shallow fried, toasted, breaded and fried, stewed and microwaved. For every food ingredient, chemical dependent processing factors are specified which will be multiplied by the contaminant concentration of an ingredient. The default value for every processing factor is set to 1, which means that by default, EN-forc assumes that processing does not influence contaminant concentrations in foods. This figure can be adjusted whenever sufficient data are available to make reasonable processing factor estimates.

So, in order to obtain total contaminant concentrations in unpackaged FoodEx2 products, EN-forc adds up the contaminant concentrations of all ingredients in proportion, taking into account the processing and fat correction factors. Contaminant concentrations in agricultural products are predicted by the environmental transfer module of EN-forc, whereas contaminant concentrations in non-agricultural and/or imported food products are added to the EN-forc model as input data (see Table 47 and Table 48 for full listings).

It is known that some food contaminants migrate from packaging materials into food products during storage (Castle, 2007). For this reason, EN-forc is extended to consider the effect of packaging on contaminant concentrations in foods. Two types of packaging factors are implemented in EN-forc: distribution and contaminant factors. Distribution factors describe the effect of packaging and the types of packaging material used on the different types of food products. In EN-forc, distribution factors are specified for six packaging types and 20 food groups (Table 46). The figures are based on data from the official Belgian Food Consumption Survey (De Vriese et al., 2005) and from other literature sources (Duffy et al., 2006a; 2006b; Poças et al., 2009).

Contaminant factors on the other hand, describe the ratio of the contaminant concentration of a packaged food product and the concentration in the same food product unpackaged or packaged in glass, which is assumed to be an inert, impermeable packaging material (Castle, 2007). If appropriate data are unavailable, contaminant factors are set to 1 by default, i.e. assuming all unpackaged food items.

In summary, in order to obtain contaminant concentrations in packaged "FoodEx2" items in EN-forc, the contaminant concentration in the unpackaged food item is multiplied by its corresponding contaminant factor. Afterwards, proportionally to the distribution of every possible packaging type, the sum is made of all these products.

Table 46: Packaging distribution factors (in %) for the 20 considered food groups (gathered from De Vriese et al., 2005; Duffy et al., 2006a; 2006b; Poças et al., 2009).

Food group	Not packaged	Plastic	Paper/ cardboard	Metal	Glass	Carton[1]
Grains and grain-based products	4.4	56.6	37.2	0.8	0.2	0.8
Vegetables and vegetable products	42.0	54.7	0.1	0.5	2.2	0.5
Starchy roots or tubers and products thereof, sugar plants	21.0	74.6	0.1	0.6	3.0	0.7
Legumes, nuts, oilseeds and spices	0	70.8	14.3	8.6	3.3	3.0
Fruit and fruit products	57.5	22.6	0	4.9	3.6	11.4
Meat and meat products	0	70.8	14.3	8.6	3.3	3.0
Fish, seafood, amphibians, reptiles and invertebrates	0	70.8	14.3	8.6	3.3	3.0
Milk and dairy products	0	17.4	1.3	0.3	0	81.0
Eggs and egg products	0.3	94.1	0.1	0.8	3.8	0.9
Sugar, confectionery and water-based sweet desserts	0	82.6	7.2	4.7	3.5	2.0
Animal and vegetable fats and oils	0	42.0	29.4	8.5	20.1	0
Fruit and vegetable juices and nectars	26.2	8.9	0	1.1	15.6	48.2
Water and water-based beverages	21.2	61.6	0	5.1	12.0	0.1
Coffee, cocoa, tea and infusions	0	94.4	0.1	0.8	3.8	0.9
Alcoholic beverages	0	0.3	0	9.1	90.0	0.6
Food products for young population	0.4	58.9	38.8	0.9	0.2	0.8
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	70.8	14.3	8.6	3.3	3.0
Composite dishes (eating outside home incl.)	80.0	14.2	2.8	1.7	0.7	0.6
Seasoning, sauces and condiments	11.7	46.9	0	10.1	7.6	23.7
Additives, flavours, baking and processing aids	0	59.2	38.9	0.9	0.2	0.8

[1] e.g. Tetra Brik®.

b) Intake and risk assessment

In EN-forc, the intake assessment of organic chemicals is implemented by means of a semi-probabilistic modelling approach: distribution of daily consumption figures are multiplied with food specific fixed contaminant concentrations and these products are summed over all foods consumed by an individual per day (Lambe, 2002). In order to do this, the food products present in the considered food consumption database have to be linked to the FoodEx2 coding system of EFSA (2011a; 2011b), as the food consumption data are not collected following the FoodEx2 coding system. Afterwards, predicted intakes are adjusted for the individuals' body weight in order to allow comparison with exposure limit values (see end of this section) on a μg kg^{-1} bodyweight basis.

To predict the distribution of the long-term usual dietary chemical intake in a population, EN-forc uses the "Mixtran" and "Distrib" algorithms from the American National Cancer Institute (NCI; Rockville, MD, USA). These algorithms are developed in SAS® (SAS Institute Inc., Cary, NC, USA) and are freely available from the NCI website[5] (Parsons et al., 2009; Tooze et al., 2010).

The EN-forc model calculates the contribution of 20 food groups to the total dietary intake of a compound. Two proportion types can be calculated: "mean proportion" and "population

[5] http://appliedresearch.cancer.gov/diet/usualintakes/macros_single.html

proportion". Mean proportion describes the average food group contribution on an individual basis and is determined by first calculating the proportion per food group for every individual and then taking the arithmetic mean of these proportions (i.e. the mean of ratios). Population proportion, on the other hand, describes the population intakes and is calculated by summing the chemical intakes for a food group over all individuals and dividing that by the total chemical intake over all food groups for all individuals (i.e. the ratio of means; Krebs-Smith et al., 1989).

Obtained chemical intake values are compared to exposure limit values to assess the risks associated with the exposure. Limit values are, for example, tolerable daily intake (TDI) values or reference dose (RfD) values and are established by authorities like EFSA, the World Health Organisation (WHO) or the American Environmental Protection Agency (US EPA).

III.2.2.2 Used data

To estimate and evaluate dietary exposure to phthalates in the Belgian adult population, the EN-forc model requires four types of input data: (1) concentration data, (2) processing and packaging factors, (3) food consumption data and (4) exposure limit values. Every data type is explained in more detail below. All model inputs are stored in an SQLite relational database system[6], allowing flexible querying and combining of heterogeneous data. Different scenarios were applied (see Section III.2.2.3).

a) Concentration data

Phthalate concentrations in agricultural products were predicted by the EN-forc model as explained in detail by Fierens et al. (2014). In summary, phthalate concentrations in agricultural products are calculated starting from observed phthalate concentrations in air, sludge, manure and concentrate and from physico-chemical property data (e.g. water solubility, vapour pressure, octanol-water partition coefficient and soil biodegradation constant). The EN-forc model uses a dynamical model simulating the soil-plant system over several growth seasons (Trapp and Legind, 2011; Vissenberg and van Grinsven, 1995). Initial concentrations of phthalates in soil were set to zero and the system was run until it reached steady-state. Phthalate concentrations in pasture were taken from a previous study (Fierens et al., 2012b), as these concentrations were overestimated by the model. The concentrations predicted by the EN-forc model for Belgian agricultural products are summarised in Table 47.

Phthalate concentrations in non-agricultural and imported products used in this study were extracted from previous Belgian monitoring studies (Fierens et al., 2012a; 2012c) and are listed in Table 48.

[6] http://www.sqlite.org/

Table 47: Phthalate concentrations in Belgian agricultural products (in µg kg-1 fw) modelled by the EN-forc model. Concentrations between parentheses are measured concentrations that were extracted from previous Belgian projects (Fierens et al., 2012a; 2012c) and used to replace over- or underestimated phthalate concentrations in the second scenario of this study.

Agricultural product	DEP	DnBP	BBP	DEHP
Animal feed				
Green crops	3.1	6.9	1.5	63
Hay	(4.0)	(13)	(19)	(24)
Pasture	(0.45)	(2.2)	(0.35)	(18)
Grains and legumes				
Barley	9.0	29	6.5	308
Bean	4.0	13	3.0	174
Maize	7.7	21	4.7	209
Pea	5.7	25	6.0	350
Oats	11	44	10	505
Rye	11	50	12	592
Spelt	9.8	36	8.1	395
Wheat	7.0	16	3.4	124
Root vegetables, onions and tubers				
Carrot	0.016 (0.30)	0.067 (3.0)	0.51 (0.40)	0.058 (12)
Celeriac	0.010 (6.0)	0.005 (0.80)	0.013 (0.50)	0.003 (20)
Onion	1.5	3.6	0.89	51
Potato	0.013 (0.20)	0.036 (3.0)	0.21 (0.10)	0.13 (24)
Radish	0.002 (0.40)	0.001 (3.0)	0.001 (0.30)	0.002 (57)
Scorzonera	0.024 (0.40)	0.14 (3.0)	0.87 (0.30)	0.063 (57)
Shallot	2.1	5.4	1.3	76
Sugar beet	0.014 (0.50)	0.008 (1.6)	0.036 (0.10)	0.003 (55)
Leafy and stalk vegetables				
Asparagus	5.4	24	5.8	348
Belgian endive	2.2	4.8	1.1	54
Broccoli	3.9	11	2.5	135
Brussels sprout	3.9	11	2.6	140
Cabbage	1.9	4.1	0.91	44
Cauliflower	3.9	11	2.5	135
Celery	2.0	4.5	1.0	49
Chervil	5.0	15	3.5	192
Chicory	2.1	4.8	1.1	54
Endive	2.2	4.8	1.1	54
Fennel	3.3	9.2	2.2	124
Leek	2.6	6.1	1.4	71
Lettuce	2.5	5.8	1.3	68
Parsley	3.9	10	2.4	126
Spinach	3.9	10	2.3	127
Fruiting vegetables				
Aubergine	0.23	0.46	0.11	6.5
Courgette	1.6	3.7	0.86	46
Cucumber	0.22	0.46	0.11	6.5
Gherkin	1.7	3.7	0.88	47
Paprika	0.31	0.64	0.15	9.1
Pumpkin	2.0	4.9	1.2	63
Tomato	0.19	0.39	0.09	5.5

Agricultural product	DEP	DnBP	BBP	DEHP
Fruits and mushrooms				
Apple	2.0	4.9	1.2	63
Cherry	6.3	40	9.5	566
Field mushroom	0.3	0.6	0.1	8.2
Gooseberry	5.0	19	4.6	266
Grape	3.4	9.7	2.3	131
Pear	2.3	5.6	1.3	73
Plum	6.6	36	8.8	513
Raspberry	4.5	16	3.7	216
Red berry	2.7	6.9	1.7	92
Rhubarb	2.0	4.8	1.2	62
Strawberry	3.0	8.5	2.1	114
Meat				
Beef	0.058	1.9	1.4	76
Chicken	0.31 (0.50)	0.42 (0.50)	176 (0.50)	0.41 (25)
Duck	0.059 (0.50)	0.38 (0.50)	19 (0.50)	0.44 (25)
Goose	0.071 (0.50)	0.79 (0.50)	24 (0.50)	0.96 (25)
Guinea fowl	0.14 (0.50)	0.16 (0.50)	86 (0.50)	0.19 (25)
Horse	0.016	0.39	0.63	1.7
Lamb	0.078	3.8	1.8	134
Pheasant	0.15 (0.50)	0.63 (0.50)	65 (0.50)	0.76 (25)
Pork	0.060	0.68	2.3	90
Quail	0.29 (0.50)	0.032 (0.50)	154 (0.50)	0.038 (25)
Rabbit	2.6 (0.50)	141 (0.50)	353 (0.10)	9276 (24)
Reindeer	0.20 (0.50)	14 (0.50)	5.5 (0.10)	1713 (24)
Turkey	0.030 (0.50)	0.82 (0.50)	11 (0.50)	0.85 (25)
Veal	0.029	1.3	1.9	52
Milk and eggs				
Cow's milk	0.034	7.4	0.59	12
Goat's milk	0.46	5.5	7.1	55
Chicken eggs	0.001	0.054	1.4	24
Liver				
Beef	0.058 (0.50)	1.9 (0.50)	1.4 (0.10)	76 (24)
Chicken	0.31 (0.50)	0.095 (0.50)	176 (0.50)	0.31 (25)
Goose liver	0.071 (0.50)	0.18 (0.50)	24 (0.50)	0.071 (25)
Pork	0.060 (0.36)	0.68 (1.1)	2.3 (2.6)	0.060 (313)
Veal	0.029 (0.50)	1.3 (0.50)	1.9 (0.10)	0.029 (24)
Water				
Tap water	0.030	0.030	0.011	0.030

Table 48: Phthalate concentrations in non-agricultural products and imported food products (in µg kg^{-1} fw) gathered from previous Belgian projects (Fierens et al., 2012a; 2012c).

Food product	DEP	DnBP	BBP	DEHP
Grains, root vegetables and legumes				
Rice	1.4	19.5	5.2	117
Shoots	2.5	3.0	1.7	7.7
Soya products	1.0	0.8	0.20	18
Starchy roots	0.20	3.0	0.10	24
Fruits and nuts				
Apricot, nectarine and peach	1.0	2.0	0.20	2.0
Banana	0.30	3.0	0.10	7.0
Citrus fruits	0.50	0.50	0.10	10
Dried fruits	1.0	2.0	6.3	12
Melon	1.8	2.0	0.20	3.6
Nuts and seeds	9.0	13	10	61
Other fruits	2.1	3.8	4.6	64
Tropical fruits	1.0	2.0	0.20	2.4
Fish and shellfish				
Crustaceans and shellfish fat	6.0	8.3	1.0	291
Crustaceans and shellfish lean	3.0	4.2	0.50	145
Crustaceans and shellfish moderate fat	1.6	3.0	0.40	29
Freshwater fish fat	0.8	8.1	1.4	154
Freshwater fish lean	0.30	3.0	0.10	7.0
Freshwater fish moderate fat	2.5	0.60	1.8	2968
Salt-water fish fat	1.4	8.0	1.4	154
Salt-water fish lean	0.90	1.0	1.5	15
Salt-water fish moderate fat	1.5	1.5	1.5	2.5
Beverages				
Bottled water	0.025	0.030	0.010	0.030
Coffee	0	0	0.008	0.26
Liqueur	0.029	0.22	0	0
Tea	0.16	0	0.025	0.14
Others				
Honey	0.50	0.50	0.10	0.50
Stock cubes and (meat) stock	16	9.0	5.0	44
Vegetable fat	10	20	29	96
Margarine	20	20	8.0	80
Meat substitute	1.0	1.0	1.0	1.0

b) Processing and packaging factors

Adequate data were available to specify processing factors for DnBP, BBP and DEHP in boiled rice, wheat (as ingredient of pasta) and potatoes, since a previous study revealed that boiling affected the levels of DnBP, BBP and DEHP in these products (Fierens et al., 2012a). Boiling factors amounted to 0.696, 0.833 and 0.314, respectively, and were calculated as the median ratio of the phthalate concentration in rice, potato and pasta after and before boiling.

Data from two previous studies (Fierens et al., 2012a; 2012c) were used to obtain packaging contaminant factors for phthalates. To do this, the measured phthalate concentrations of the different food products were divided into groups according to the 20 food categories and six packaging types considered in the EN-forc model (Table 46). Subsequently, Tukey's Honest Significant Difference tests were performed in order to investigate if phthalate concentrations in a food group unpackaged or packaged in glass differed significantly from phthalate concentrations in the same

food group, but packaged in another packaging material. Statistical significance was based on the level of $p < 0.10$. For significant differences, packaging contaminant factors were calculated as the median of the ratios of phthalate concentrations in a food group packaged in a certain packaging material and the concentration in the food group unpackaged or packaged in glass. The following packaging contaminant factors were obtained: 3.48 for DEHP and 1.02 for DnBP in vegetables, starchy roots and tubers, legumes, fruits and eggs packaged in plastic; 2.23 for BBP in fats and oils and seasonings packaged in metal; 15.5 for DnBP in juices, water(-based beverages), coffee, cocoa and tea packaged in carton (e.g. Tetra Brik®) and 9.18 for DnBP in milk and dairy products packaged in paper or cardboard.

c) Food consumption data

The latest Belgian National Food Consumption Survey conducted in 2004 was used in this study to calculated dietary exposure to phthalates (Devriese et al., 2006). In this survey, food consumption data were collected for a representative sample of the Belgian adult population between 15 and 98 years old, with a median age of 57. The design of the Belgian National Food Consumption Survey followed the recommendations of the European Food Consumption Survey Method project (Brussaard et al., 2002a; 2002b; De Henauw et al., 2002). Food consumption data were collected by means of two repeated non-consecutive 24 hour recalls (face to face). The survey covered a full year, with interviews distributed equally over the seasons and days of the week. During the 24 hour recall interviews, participants reported types and quantities of all foods and beverages consumed over the preceding day. The 24 hour recall interviews were carried out using the validated EPIC-SOFT program to obtain standardised interviews (Slimani and Valsta, 2002). This software program allows obtaining very detailed descriptions and quantifications of foods, recipes and supplements consumed and automatically generates remarks (e.g. reporting of extremely low or high portion sizes) for the interviewers that had to be checked before finishing the 24 hour recall interviews. Complete data were available for 3,061 adults: 1,523 women and 1,538 men. More details about the methodology and the population characteristics of this survey can be found elsewhere (De Vriese et al., 2005; Temme et al., 2010).

As stated in Section III.2.2.1, all food products present in the considered food consumption database (Devriese et al., 2006) were linked to the FoodEx2 coding system of EFSA (2011a; 2011b). For the Belgian National Food Consumption database, 743 FoodEx2 codes were involved.

d) Exposure limit values

TDI values for DnBP, BBP and DEHP were established by EFSA (2005a; 2005b; 2005c) and amounted to 10, 500 and 50 µg kg^{-1} bw day^{-1}, respectively. For DEP, a TDI value of 5,000 µg kg^{-1} bw day^{-1} was established by WHO (2003).

III.2.2.3 Scenarios

The validation of the environmental transfer module of the EN-forc model showed that estimated concentrations of some phthalates in some agricultural products differed more than one orde of magnitude compared to measured phthalate levels (Fierens et al., 2014). Since the use of inaccurate data might have implications on the risk assessment results, two exposure scenarios were considered in this study. In the first scenario, modelled EN-forc concentrations of phthalates in all considered Belgian agricultural products were used to assess dietary phthalate intake in the Belgian adult population. In the second scenario, under- or overestimated EN-forc concentrations were replaced by measured concentrations obtained in previous Belgian monitoring studies (Fierens et al., 2012a; 2012c). The concentrations, replacing the predicted EN-forc concentrations, can be found between parentheses in Table 47.

In both scenarios, age and gender were considered as covariates to estimate the distribution of the long-term average dietary phthalate intake of the Belgian adult population. This way, intake values were obtained for the following subgroups: total population, males, females, adolescents (15 – 18 years old), “working age” population (19 - 65 years old), elderly (66 - 75 years old) and very elderly (76 - 98 years old). To test for gender and age differences, a two-way ANOVA model was used for every phthalate compound. For all considered phthalates, the interaction between gender and age was not significant, which lead to a model with only two main effects (i.e. gender and age). To investigate if the average intake estimates of the considered subpopulations were significantly different from each other, the Tukey’s Honest Significant Difference (Tukey’s HSD) test was used. Both statistical tests were carried out using R version 2.15.2 (R Development Core Team, 2011). Statistical significance was based on the level of $p < 0.01$.

Since the main interest of this study goes to the upper end of the intake distribution, for each population group, the 50th (P50), 95th (P95) and 99th percentiles (P99) of the long-term intake distributions of the four considered phthalates are reported. The contributions of the 20 considered food groups to the total dietary phthalate intakes were calculated as population proportions.

III.2.3 Results

III.2.3.1 Intake distribution

Dietary intake distributions of DEP, DnBP, BBP and DEHP for the total Belgian adult population are shown for the two considered exposure scenarios in Figure 15. As can be noticed, replacing under- or overestimated EN-forc concentrations by measured concentrations only affected the intake distribution of BBP. Intake rates of BBP calculated in the first scenario were about two times higher than those calculated in the second scenario. For instance, P50, P95 and P99 intake rates for BBP in scenario 1 amounted to 0.081, 0.115 and 0.203 µg kg^{-1} bw day^{-1} compared to 0.040, 0.069 and 0.086 µg kg^{-1} bw day^{-1} in scenario 2, respectively.

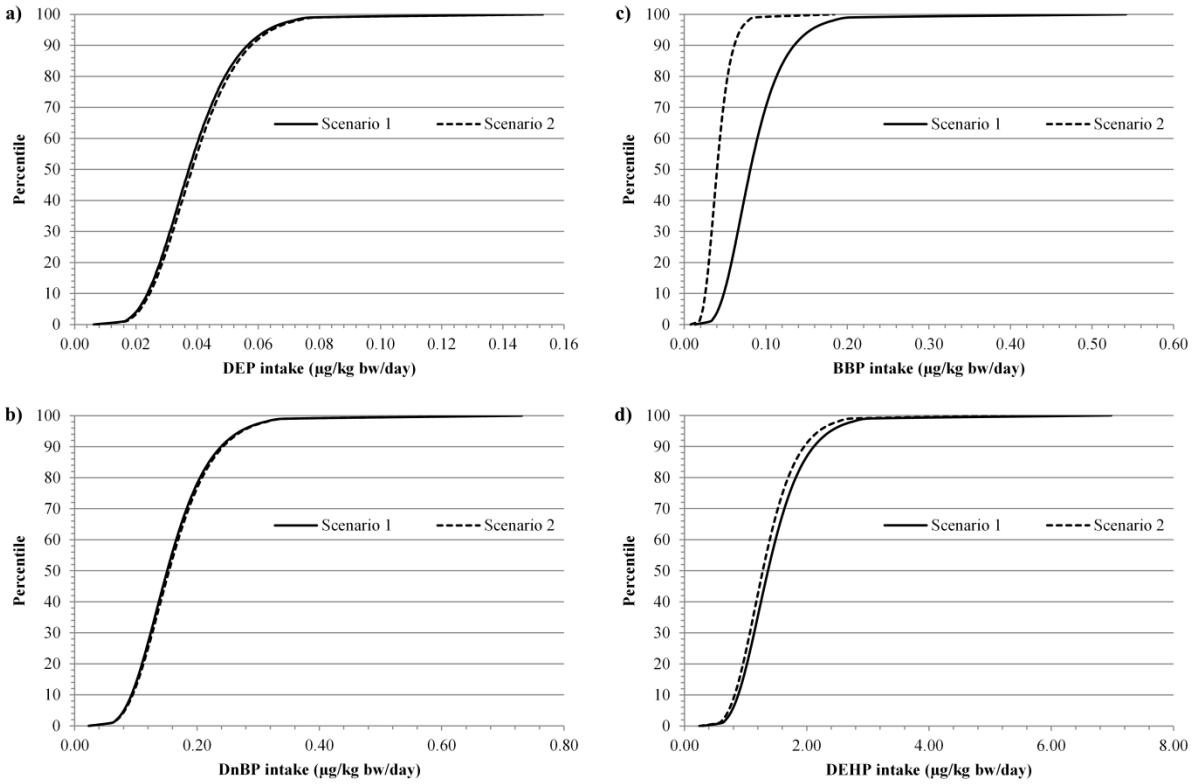

Figure 15: Dietary intake distributions of DEP (a), DnBP (b), BBP (c) and DEHP (d) for the total Belgian adult population considering two semi-probabilistic exposure scenarios per phthalate compound. Scenario 1: using estimated phthalate concentrations in all considered Belgian agricultural products; Scenario 2: replacing under- or overestimated phthalate concentrations in agricultural products by measured concentrations from previous Belgian projects (Fierens et al., 2012a; 2012c).

Table 49 summarises the predicted average, P50, P95 and P99 intake value of every phthalate compound of the first scenario for the total Belgian adult population and the considered subgroups. Predicted exposure values were highest for DEHP, followed by DnBP, BBP and DEP. Regarding dietary exposure to DEP, DnBP and BBP, Belgian men were significantly more exposed than Belgian women. Although not statistically significant (p = 0.027), predicted dietary exposure values for DEHP were also higher in Belgian men than in women. With regard to the considered age categories, predicted exposure rates of DEP, DnBP and BBP decreased with age. For DEP, predicted average exposure rates were significantly higher in young adults (15 – 18 years old) than in very elderly (76 – 98 years old); for DnBP and BBP, this difference was of borderline significance (*p*-values of 0.014 and 0.019, respectively). In contrast to these three phthalates, predicted average exposure rates of DEHP were significantly lower in young adults (15 – 18 years old) than in the very elderly group (76 – 98 years old). Comparing the calculated dietary exposure values of the phthalate compounds with their corresponding TDI values (Table 49), revealed that for none of the investigated compounds and population groups, exposure limit values were exceeded.

Table 49: Estimated phthalate intake values (average, P50, P95 and P99) of the first scenario for the total Belgian adult population and for subgroups of this population (in µg kg-1 bw day-1). Corresponding tolerable daily intake (TDI) values are mentioned for comparison.

Population group	N	Average	P50	P95	P99
DEP		*TDI = 5,000 µg kg^{-1} bw day^{-1}*			
Total	3061	0.039	0.037	0.063	0.077
Male	1538	0.041 *	0.039	0.066	0.080
Female	1523	0.037	0.036	0.060	0.074
15 - 18 y.	574	0.043 a	0.041	0.069	0.083
19 - 65 y.	1280	0.040 b	0.038	0.064	0.078
66 - 75 y.	509	0.037 b,c	0.036	0.061	0.074
76 - 98 y.	698	0.036 c	0.034	0.058	0.071
DnBP		*TDI = 10 µg kg^{-1} bw day^{-1}*			
Total	3061	0.160	0.151	0.271	0.340
Male	1538	0.166 *	0.157	0.280	0.351
Female	1523	0.155	0.146	0.262	0.330
15 - 18 y.	574	0.173 a	0.163	0.291	0.365
19 - 65 y.	1280	0.158 b	0.149	0.267	0.335
66 - 75 y.	509	0.158 b	0.149	0.267	0.333
76 - 98 y.	698	0.157 a,b	0.149	0.265	0.335
BBP		*TDI = 500 µg kg^{-1} bw day^{-1}*			
Total	3061	0.088	0.081	0.155	0.203
Male	1538	0.093 *	0.087	0.165	0.215
Female	1523	0.082	0.076	0.145	0.188
15 - 18 y.	574	0.098 a	0.091	0.173	0.225
19 - 65 y.	1280	0.090 a	0.083	0.158	0.206
66 - 75 y.	509	0.081 a	0.075	0.143	0.184
76 - 98 y.	698	0.081 a	0.075	0.143	0.187
DEHP		*TDI = 50 µg kg^{-1} bw day^{-1}*			
Total	3061	1.45	1.37	2.39	3.01
Male	1538	1.52	1.44	2.51	3.15
Female	1523	1.38	1.30	2.27	2.85
15 - 18 y.	574	1.36 a	1.29	2.25	2.83
19 - 65 y.	1280	1.42 a,b	1.34	2.34	2.95
66 - 75 y.	509	1.56 b	1.47	2.58	3.22
76 - 98 y.	698	1.49 b	1.41	2.45	3.10

N: number of participants; * Males significantly different from females ($p < 0.01$); a,b,c These age categories are significantly different from each other ($p < 0.01$).

III.2.3.2 Contribution of food groups

The contribution of each of the 20 considered food groups to the total dietary intake of DEP, DnBP, BBP and DEHP in the Belgian adult population is illustrated in Figure 16. Results are shown only for the first scenario (i.e. with model predictions for all product concentrations). For DEP, grains and grain-based products (39.5%) contributed the most to the total dietary DEP intake, followed by fruit and fruit products (8.9%) and alcoholic beverages (8.2%). With respect to DnBP, the three main contributors to dietary exposure were milk and dairy products (24.9%), grains and grain-based products (24.2%) and animal and vegetable fats and oils (12.4%). Dietary exposure to BBP in Belgian adults mainly originated from the intake of contaminated meat and meat products (55.6%), grains and grain-based products (9.4%) and composite dishes (6.3%). For DEHP, meat and meat products (18.7%) was the main contributor to dietary DEHP exposure, followed by fruit and fruit products (18.4%) and grains and grain-based products (15.7%). For DEP and DnBP, the contribution percentages of the top three food groups did not differ very much between scenario 1 and scenario

2; for BBP and DEHP on the other hand, dissimilarities were noticed. In scenario 2, the three main contributors to dietary BBP exposure were grains and grain-based products (22.8%), sugar, confectionery and water-based sweet desserts (10.9%) and meat and meat products (9.9%); the top three for DEHP consisted of grain and grain-based products (18.7%), fruit and fruit product (16.2%) and vegetables and vegetable products (13.4%).

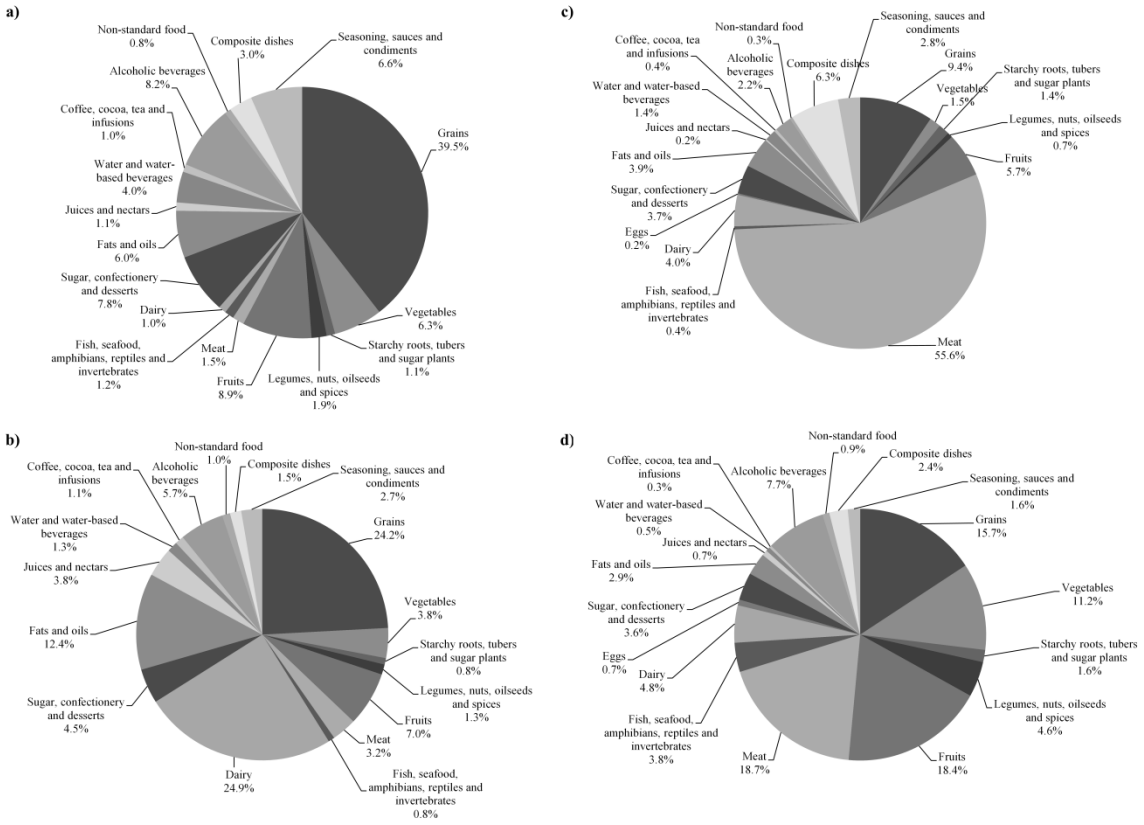

Figure 16: Contribution of the 20 considered food groups to the total dietary intake of DEP (a), DnBP (b), BBP (c) and DEHP (d) in the Belgian adult population (first scenario results).

III.2.4 Discussion

This study estimated and evaluated the long-term dietary intake of DEP, DnBP, BBP and DEHP in the Belgian adult population. The highest predicted exposures were found for DEHP, followed by DnBP, BBP and DEP (Table 49). In general, Belgian men were more exposed to phthalates than Belgian women and – with the exception of DEHP – a declining exposure with increasing age could be observed (Table 49). A possible reason for the dissimilarity between males and females is that, on average, Belgian men consume more grain-based products (bread, rice, pasta, breakfast cereals, etc.), meat, dairy products and alcoholic beverages than Belgian women (Vandevijvere et al., 2008), which are all top three contributors to the dietary exposure of at least one of the considered phthalates (Figure 16). Considering that older Belgian people, on average, consume less grain-based products than young Belgian people (Vandevijvere et al., 2008) and that this food group is the main or second main contributor to the dietary exposure of DEP, DnBP and BBP (Figure 16) may explain why lower exposure estimates were predicted for DEP, DnBP and BBP in the very elderly than in the

young adult group. The reason why older Belgian people were significantly higher exposed to DEHP through food ingestion than Belgian young adults, may be that grain-based products were only the third main contributor to the total dietary intake of DEHP (Figure 16). Moreover, the second main contributor for DEHP was fruit and fruit products, of which was observed that the average consumption by older Belgian age categories is higher than by younger categories (Vandevijvere et al., 2008).

The fact that Belgian men and young adults were generally predicted to be more exposed to phthalates through dietary intake is noteworthy, since several studies indicated that phthalates are associated with negative health outcomes in the male reproductive system (Hauser and Calafat, 2005; Heudorf et al., 2007; Kamrin, 2009; Meeker et al., 2009). Fortunately, all predicted exposure rates were far below the available exposure limit values (Table 49): the 99th intake percentiles of DEP and BBP were less than 1% of their corresponding TDI values (EFSA, 2005b; WHO, 2003) and the P99 intake values of DnBP and DEHP were between 3.3 and 3.7% and between 5.7 and 6.4% of the established TDI values (EFSA, 2005a; 2005c), respectively. This is reassuring since adults are also exposed to these phthalates via other contamination pathways. This is especially the case for DEP: more than 80% of the exposure to DEP is caused by the dermal application of personal care products and only 10% is derived from food ingestion (Wormuth et al., 2006).

III.2.4.1 Comparison with other dietary intake assessments

The predicted intake results for DEP, DnBP, BBP and DEHP in the total Belgian population of this study were compared with the dietary intake assessment results of the Belgian PHTAL project (Sioen et al., 2012). In Figure 17, the predicted P50 and P95 exposure rates for the four considered compounds from both studies are shown (first scenario results for the current study; upper bound probabilistic results considering preparation for the PHTAL project). As can be noticed from Figure 17, P50 as well as P95 intake rates calculated by EN-forc agreed well with the intake rates calculated by Sioen et al. (2012). In the PHTAL project, the top three of the food groups contributing most to the long-term intake of DEP, DnBP, BBP and DEHP were determined. For DEP, the top three consisted of fruits (20.9%), bread (11.8%) and pasta and other grains (10.0%). For DnBP, bread (19.7%), processed meat (14.0%) and biscuits and cakes (10.7%) were the major sources to dietary exposure. The top three of food groups contributing most to the dietary exposure of BBP were fruits (28.3%), olive oil (14.9%) and alcoholic drinks (14.0%) and for DEHP, the top three consisted of bread (31.4%), fruits (8.6%) and fresh meat (8.1%) (Sioen et al., 2012). As can be observed from Figure 16, most of these food groups are also main contributors to the dietary intake of DEP, DnBP, BBP and DEHP in this study. Since phthalates are lipophilic compounds, it was rather unexpected that in both studies – besides meat products, dairy products and vegetable oils – also food groups such as fruit and fruit products, grain-based products and alcoholic beverages were determined to be main contributors to the dietary exposure of DEP, DnBP, BBP and DEHP. The same observation was more or less done in a recent British survey based on total diet samples, in which bread, miscellaneous cereals, green vegetables, beverages, fish, meat and poultry were determined to be the major contributors to the dietary exposure to phthalates (COT, 2011). With the exception of alcoholic beverages, all these food groups are foods that are consumed frequently and in high amounts. So, decreasing the contamination of these types of foods would almost certainly have a considerable effect on the dietary intake of phthalates. Possible sources for phthalate contamination in these types of foods may be environmental transfer (e.g. deposition from air, soil-root uptake and use of pesticides) as

well as migration from food contact materials used during production and storage like conveyor belts and packaging materials, respectively (Cao, 2010; Dickson-Spillmann et al., 2009; Groshart and Okkerman, 2000). Another remarkable aspect is that most of the important contributing food groups are "healthy" food products indicating that especially people with a healthy lifestyle are exposed to phthalates. The same conclusion was made by Dickson-Spillmann et al. (2009) among others. This research group observed higher DEHP, BBP and DEP exposure rates in conscious consumers compared to others.

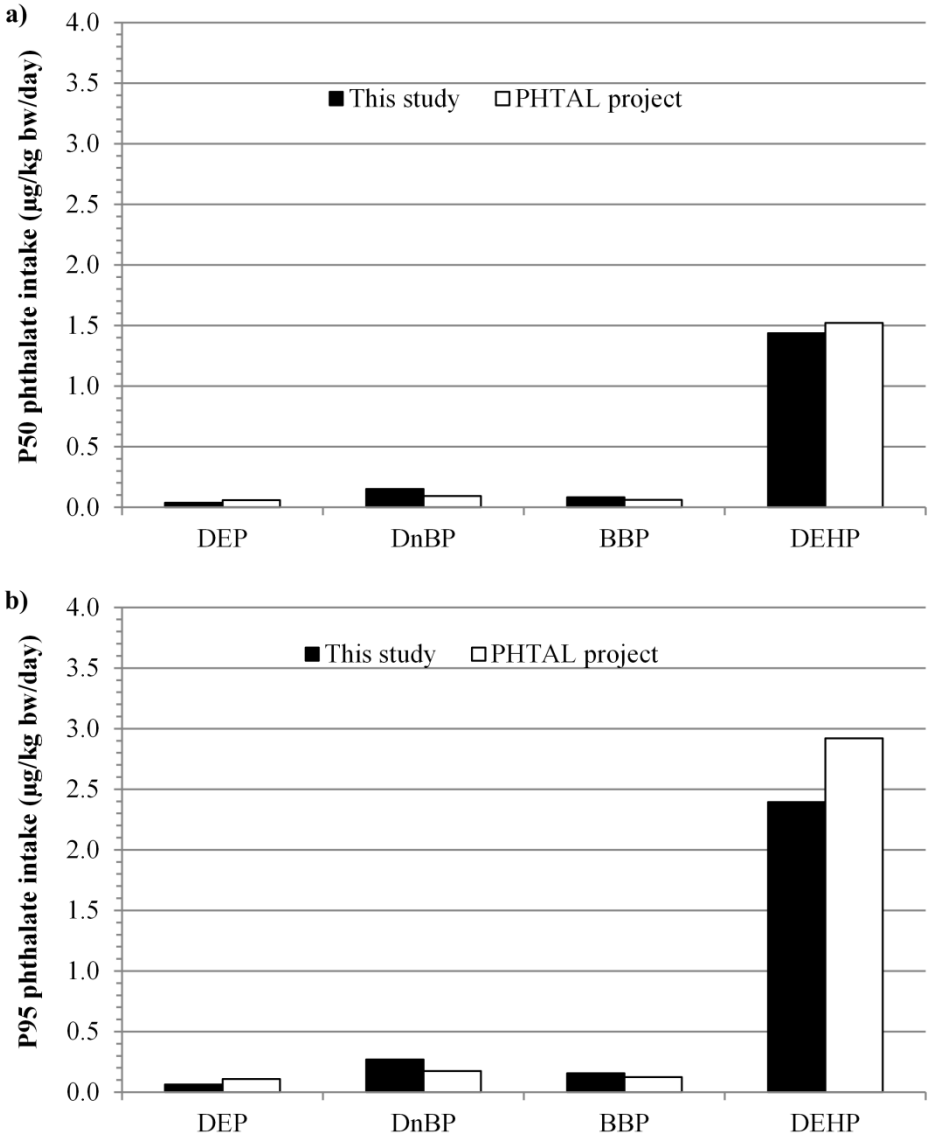

Figure 17: Comparison of estimated phthalate intake values (P50 (a) and P95 (b); in µg kg-1 bw day-1) for the total Belgian adult population of this study (first scenario) with those of the PHTAL project (Sioen et al., 2012; upper bound probabilistic scenario considering preparation).

Franco et al. (2007) used the EUSES model to predict DnBP and DEHP exposure in the Dutch adult population. EUSES stands for "*Union System for the Evaluation of Substances"* and is a multimedia fate and exposure model commissioned as the preferred modelling tool for risk assessments in the European Union (Vermeire et al., 2005). As imposed by the EUSES model, Franco et al. (2007) considered the following exposure routes for DnBP and DEHP: inhalation of (outdoor) air, ingestion of drinking water and ingestion of food (beef, dairy, fish, root crops and leaf crops). Median total daily intakes were predicted to be 0.21 and 0.68 µg kg^{-1} bw day^{-1} for DnBP and DEHP, respectively. Since the contribution of air inhalation to the total intake of DnBP and DEHP only accounted for about 1%, these median values can be regarded as predicted dietary intake values for DnBP and DEHP. Although only five food types were considered by the EUSES model, predicted median intake values in the study of Franco et al. (2007) were remarkably within the same order of magnitude as the P50 intake values calculated in this study (Table 4). However, this unexpected observation may be explained. Since the last decade, legislation about the use of DnBP and DEHP in Europe has been tightened, e.g. their use in toys, childcare articles and cosmetic products has been restricted (Official Journal of the European Union, 2005; SCCP, 2007) with a declined usage of these two phthalates by European industries as a consequence (ECPI, 2010). So, the fact that the intake results of the study of Franco et al. (2007), in which input data were gathered from studies conducted in 2003 and before, and the current study were unexpectedly comparable to each other, is most likely owing to an underestimation of the DnBP and DEHP exposure by the EUSES model. As a matter of fact, this hypothesis has already been confirmed in the publication of Franco et al. (2007) itself.

Heinemeyer and co-workers (2013) estimated dietary exposure to DEHP in the German adult population (14-80 years old). In their study, two estimation methods were used: a deterministic and a probabilistic approach. In the deterministic exposure estimation method, average food consumption rates were combined with average and median concentrations of DEHP in 37 food product categories. According to this deterministic approach, dietary intake to DEHP in German adults amounted to 9.3 and 3.6 µg kg^{-1} bw day^{-1} by using average and median DEHP concentration levels, respectively. When calculated probabilistically, the average, median and P95 dietary exposure to DEHP amounted to 14.0, 10.2 and 28.6 µg kg^{-1} bw day^{-1}, respectively. The food categories contributing most to the dietary exposure to DEHP in German adults were: bread/rolls, spicy dressings, butter, vegetables, coffee/tea and fine bakery wares. Compared to this study, German adults seem to be higher exposed to DEHP through the diet than Belgian adults. However, as also indicated by Heinemeyer et al. (2013), it should be mentioned that the DEHP exposure rates in the German study may have been overestimated due to the use of rather old (i.e. from 1990 onwards) concentration data of DEHP in foods.

Wormuth et al. (2006) investigated exposure sources for eight frequently used phthalates (including DEP, DnBP, BBP and DEHP) in different age groups of the European population. Exposure pathways considered in this study were food consumption, dust and soil ingestion, mouthing (for children), ingestion of personal care products, dermal exposure and inhalation. With regard to food consumption, scenarios were based on the amount of food products consumed daily, the fraction of consumers eating these foods on a regular basis and phthalate concentrations measured in the food products. Intake estimations revealed that for DEP, only 10% of the total DEP intake in adults was derived from food ingestion. For DnBP, food contributed for 80 and 90% to the total intake in female and male adults, respectively. For BBP and DEHP, food intake was responsible for 60 and 100% of the

total intake, respectively. Given these contribution percentages, P50 (P95) dietary intake values predicted in female adults were 0.14 (6.5) µg kg^{-1} bw day^{-1} for DEP, 2.8 (30.9) µg kg^{-1} bw day^{-1} for DnBP, 0.16 (0.99) µg kg^{-1} bw day^{-1} for BBP and 2.5 (14.7) µg kg^{-1} bw day^{-1} for DEHP. In male adults, predicted P50 (P95) values amounted to 0.12 (5.1), 3.3 (16.7), 0.19 (1.1) and 2.8 (16.3) µg kg^{-1} bw day^{-1}, respectively. Compared to this study (Table 49), the dietary intake results for DEP and DnBP calculated by Wormuth et al. (2006) were much higher (i.e. a difference of more than one to two orders of magnitude). On the other hand, similar intake levels (i.e. within one order of magnitude) for BBP and DEHP were observed in the two studies. Dissimilarities in phthalate exposure estimates between the two studies may be owing to the origin of the phthalate concentration data used by Wormuth et al. (2006): Wormuth et al. (2006) used European as well as American and Asian data to assess dietary phthalate intake in the European population. Since legislations about the use of phthalates may differ between the European Union and non-European countries (Wordsworth, 2007), it is possible that the use of non-European data by Wormuth et al. (2006) may have led to overpredictions.

Clark et al. (2011) modelled total human exposure to seven phthalates (DEP, DnBP, BBP and DEHP included) by using Canadian intake rates and concentration data from the most recent version of the American Chemistry Council database. Considered pathways in this study were inhalation and the ingestion of food, drinking water, soil and dust. Exposure via food ingestion – as calculated by combining food intake rates with measured phthalate concentrations in food – contributed for 57, 75, 72.5 and 95% to the total adult exposure of DEP, DnBP, BBP and DEHP, respectively. Considering these contribution percentages, P50 dietary intakes calculated by Clark et al. (2011) amounted to 0.26 µg kg^{-1} bw day^{-1} for DEP, 0.90 µg kg^{-1} bw day^{-1} for DnBP, 0.36 µg kg^{-1} bw day^{-1} for BBP and 10.5 µg kg^{-1} bw day^{-1} for DEHP. Corresponding P95 intake rates were 0.57, 2.3, 1.0 and 29.5 µg kg^{-1} bw day^{-1}, respectively. Both P50 and P95 exposure values of the four considered phthalates calculated by Clark et al. (2011) were about one order of magnitude higher than the results predicted in this study (Table 49), which may again be owing to the origin of data used in the study of Clark et al. (2011) and this study (i.e. American and Canadian data compared to European data, respectively).

III.2.4.2 Limitations and recommendations for future research

This study showed that the EN-forc model can be useful as a semi-probabilistic modelling tool for predicting and evaluating the long-term dietary intake of organic chemicals in a human population. However, some limitations and recommendations for future research have to be discussed.

First of all, comparing the contributions of the 20 considered food groups to the total dietary exposure of BBP and DEHP in scenario 1 with their contributions in scenario 2, revealed that using inaccurate data did have an effect on the intake assessment results. BBP concentrations in meat (especially poultry) were overestimated by the EN-forc model, which resulted in an overestimation of the contribution of meat and meat products to the total dietary exposure of BBP in scenario 1 (55.6%; using only modelled concentrations in agricultural products) compared to scenario 2 (9.9%; replacing under- or overestimated concentrations by measured levels). Although in a minor degree, the opposite was observed for DEHP in vegetables and vegetable products. DEHP concentrations in some food products belonging to this food group (i.e. root vegetables and tubers) were underestimated by the EN-forc model and thus led to a (small) underestimation of the contribution of vegetables and vegetable products to the dietary exposure to DEHP, namely 11.2% in scenario 1

compared to 13.4% in scenario 2. The fact that phthalate concentrations in some agricultural products were under- or overestimated by the EN-forc model, has already been discussed in detail by Fierens et al. (2014) who stressed the need for the development of suitable transfer models for organic contaminants like phthalates in poultry and root and tuberous vegetables.

EN-forc predicts contaminant concentrations in 1,908 FoodEx2 products spread over 20 different food groups. These concentrations are based on predicted phthalate concentrations in about 100 basic agricultural products and on measured phthalate concentrations in about 30 non-agricultural (imported) products. Compared to the number of food items that is present on the European market, this number is rather limited and may not cover the whole food product spectrum. However, this number is still extensive when e.g. compared to the study of Franco et al. (2007), in which only five food products (i.e. beef, dairy, fish, root crops and leaf crops) were considered by using the EUSES model.

To obtain packaging distribution factors to implement in the EN-forc model, information was lacking in the Belgian National Food Consumption Survey (Devriese et al., 2006) for several food groups. To meet this gap, information from other European surveys (Duffy et al., 2006a; 2006b; Poças et al., 2009) was taken, but this was only possible by assuming that the packaging distribution of food groups in every European country is the same.

The processing and/or packaging factors that are currently implemented in the EN-forc model, are not predicted but based on monitoring results (Fierens et al., 2012a; 2012c). Since such data are often not adequately available, it is desirable that models would be developed with an acceptable level of complexity to implement in EN-forc that could predict such factors.

Besides for phthalates, the EN-forc model has also been used for the prediction of the environmental transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans into agricultural products (Fierens et al., 2014). When enough data regarding the effect of packaging and processing and regarding concentrations in non-agricultural media are at hand, the EN-forc model could also be used to predict dietary exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans in Belgian adults. Exposure to other chemical classes could also be considered in the future, but again, this is only possible when enough high quality data are available to specify the model inputs (e.g. physico-chemical properties, background concentration data, processing factors, and so on). Additionally, the EN-forc model also have to be validated for these chemical classes with chemicals having a similar chemical and biological behaviour first.

Lastly, it could be considered to make a fully probabilistic dietary exposure assessment tool of the EN-forc model. Advantages of this kind of extension would be that concentration ranges of contaminants in food products would be predicted and that it would be possible to test the sensitivity and accuracy of the model and its input data.

III.2.5 Conclusions

The semi-probabilistic dietary intake assessment of four phthalates for the Belgian adult population carried out in this study revealed that DEHP had the highest intake estimates, followed by DnBP, BBP and DEP. Belgian men and young adults were generally more exposed to phthalates than Belgian women and the elderly. Nevertheless, predicted dietary intake rates for all four investigated phthalates were far below the corresponding TDI values (i.e. P99 intake values were 6.4% of the TDI at most), which is reassuring because exposure to these phthalates also occurs via other pathways. The food groups contributing the most to the dietary exposure of DEP, DnBP, BBP and DEHP were, respectively, grains and grain-based products, milk and dairy products, meat and meat products and meat and meat products. Comparing the predicted intake results with intake estimates from other studies showed that the extended version of the EN-forc model is a suitable tool for the estimation and evaluation of the long-term dietary intake of phthalates in humans.

IV.Discussion

During the last decades, phthalates have been used quite extensively in a wide range of user applications. Due to this widespread use, phthalates contaminated the (indoor and outdoor) environment as well as the human food chain (see Chapter I.2 and I.3). Because phthalates and their metabolites have been reported to cause negative health effects in humans (see Section I.4.3), even when exposure occurs at background doses, it is important to determine the most important exposure pathways to phthalates. For several phthalates, especially DEHP, numerous foreign studies revealed that dietary intake is one of the major exposure routes (see Section I.4.1). Unfortunately, with respect to Belgium, data on dietary phthalate intake were not available at the time this PhD study started.

To fill this knowledge gap, this PhD aimed to estimate and evaluate the dietary exposure of the Belgian adult population to eight phthalate compounds: DMP, DEP, DiBP, DnBP, BBP, DCHP, DEHP and DnOP. In order to achieve this goal, eight specific research objectives were determined (see Chapter I.7). These specific objectives can be further subdivided into three main topics, namely (1) analysing the occurrence of phthalates in foods, (2) modelling the occurrence of phthalates in foods and (3) estimating dietary phthalate exposure in the Belgian adult population. In the first chapter of this general discussion, a summary can be found of the major results and discussion points obtained for each of these three topics.

The next three chapters of this general discussion put the topic of this PhD – phthalates in the food chain – into a broader perspective. The first of these three chapters is dedicated to the relevance of this PhD to public health. In the second chapter, suggestions for policy development regarding legislation about food products and food contact materials are described. Possible alternative substances to phthalates are elaborated in this chapter as well. Recommendations for further research are highlighted in the third chapter. Here, a distinction is made between recommendations for improving the analytical measurement procedure, model development and exposure assessment.

The general discussion part finishes with some concluding remarks, which can be found in the fifth chapter.

IV.1 Summary of major results and discussion points

IV.1.1 Analysing the occurrence of phthalates in foods

Within the PHTAL project, an analytical procedure was developed and validated for the determination of eight phthalate compounds (DMP, DEP, DiBP, DnBP, BBP, DCHP, DEHP and DnOP) in food products and packaging materials present on the Belgian market by means of gas chromatography - low resolution - mass spectrometry with electron impact ionisation (GC-EI-MS; see Chapter II.1). Depending on the extraction technique used, a distinction was made between low-fat foods, high-fat foods, aqueous-based beverages and packaging materials.

Due to the omnipresence of phthalates in the laboratory environment, a sample handling protocol was set up to reduce the risk of contamination during sample preparation and analysis (see Chapter II.1). For instance, all laboratory glassware was heated at 450 °C for at least four hours and covered with aluminium foil in advance. Also, before use, all glassware, syringes, spatula and other laboratory equipment were rinsed carefully with dichloromethane. By following these guidelines, the process of analysing phthalates became expensive and time consuming. The major consequence of this was that the number of samples analysed during this PhD in order to determine specific exposure pathways was rather limited. Due to the limited number of samples, the analytical data obtained were often insufficient to allow powerful statistical analyses. However, this did not prevent that in this dissertation a number of interesting trends were identified and several hypotheses were formulated regarding possible contamination pathways for phthalates in the Belgian food chain, which will be discussed in the following paragraphs. As such, this PhD thesis may serve as a basis for future research aiming to study particular contamination pathways more into detail (see also Chapter IV.4).

In the PHTAL project, the occurrence of phthalates in 621 different food and packaging samples was investigated. These samples were collected and analysed during two measurement campaigns: a first screening campaign (n=400; see Chapter II.1) and a second more specific campaign (n=221; see Chapter II.2). In both campaigns, DEHP was the most detected phthalate compound, followed by DiBP, DnBP and BBP. DMP, DEP, DCHP and DnOP, on the contrary, were rarely present in the investigated samples. In general, levels of DEHP were the highest of all studied phthalates in every investigated food group, notwithstanding some exceptions in particular food groups with high concentrations of other phthalates. For example, cereals and cereal products like rice and flour were highly contaminated with DiBP and high concentrations of BBP were observed in grape seed oil. The lowest phthalate concentrations were observed in milk and milk beverages, baby food, vegetarian food, eggs and aqueous-based beverages. Phthalate levels determined in the considered Belgian food samples were generally comparable with levels observed in other recent studies (Bradley et al., 2013a; Peters, 2006; Pfördt, 2004; Poças et al., 2010; Schecter et al., 2013; Tsumura et al., 2001c; 2002b). In packaging materials, especially in cardboard, phthalate contamination was primarily due to the presence of DiBP. This is not surprising, since DiBP is often used as additive in printing inks and adhesives of food contact materials to improve surface adhesion, flexibility and wrinkle resistance. Furthermore, traces of DiBP can also be present in cardboard due to the use of recycled materials (BfR, 2007; Bradley, 2012; CDC, 2009; Gartner et al., 2009).

Besides obtaining accurate data of phthalates in various types of food products and packaging materials present on the Belgian market, the PHTAL project also aimed to get a clear understanding of possible contamination pathways for phthalates in the Belgian food chain (see Chapter II.2). For instance, concentration profiles of five food products (i.e. apple, semi-soft cheese, bread, salami and soft goat's cheese) were conducted by analysing phthalates in samples taken at different places of the respective foods. Within the investigated bread, salami and cheese samples, phthalates were observed to be distributed uniformly. Since all these food products had in common that they were processed, it was most likely that phthalates entered these food items during one or more of the processing steps (e.g. baking, ingredient mixing or pasteurisation) rather than as a result of packaging migration. The concentration profile of apple on the other hand, revealed that the skin contained more DiBP, DnBP, BBP and DEHP than the flesh of the investigated apple. Since apple is not processed, other sources have to be responsible for the introduction of phthalates in this food product. Examples of such sources might be the use of phthalate containing pesticides and/or phthalate containing wax coatings on apples (Lin and Zhao, 2007; Navarro-Tarazaga et al., 2008; Yin et al., 2011).

High phthalate concentrations – especially DEHP – were observed in bread during the first measurement campaign of the PHTAL project (see Chapter II.1). Considering that bread is almost daily consumed by the Belgian population, it was decided to analyse additional bread samples during the second measurement campaign (see Chapter II.2). In this second campaign, the effect of the type of packaging, flour, location, form and the contact time with the bread bag on the occurrence of phthalates in 43 purchased bread samples was studied. The most remarkable result of this investigation was that concentrations of DiBP, DnBP, BBP and DEHP – the four main detected phthalates – in bread were mainly depending on the specific situation of the bakery, which resulted in a wide variety on phthalate concentrations observed in the investigated bread samples. An explanation for these wide concentration ranges may be the use of different ingredients (e.g. yeast, bread enhancer and flour), contact materials (e.g. oven and baking trays), bread bags, and so on by the investigated bakeries. Considering that people often buy bread at the same bakery, there is a risk that these people may be highly exposed to phthalates as a result of the regular consumption of contaminated bread.

To investigate contamination pathways more in depth, one type of food chain was selected for a more detailed analysis in this PhD project. To choose which Belgian food chain would be studied, scientific publications and reports were consulted as the screening campaign of the PHTAL project was not finalised yet at that time. The choice was made to investigate a Belgian contemporary milk production chain and this for several reasons. First of all, milk and dairy products were considered to be an important food group for the Belgian population, especially for young children (Vanhauwaert, 2012). Secondly, since phthalates are lipophilic, they tend to concentrate more in high-fat foods like cream, butter and cheese than in low-fat foods (Zhu et al., 2010). Lastly, as observed by Clark et al. (2003a) for the Canadian population, milk and dairy products seem to be the main contributors to the daily dietary exposure to phthalates. During this milk chain campaign, samples of milk and dairy products were collected at farm (see Chapter II.4), industry and retail level (see Chapter II.5).

At five farms located in the northeast of Belgium, samples of mechanically obtained raw cow's milk were collected, twice during winter and twice during summer. The phthalate levels analysed in these

samples seemed to be both farm and season dependent. Therefore, at two of the five farms, additional samples of manually obtained milk, feed, soil and groundwater were gathered in order to identify contamination sources in more detail. At the two considered farms, DiBP and DEHP exposure in cows (with resulting DiBP/DEHP contamination in milk) mainly originated from the ingestion of contaminated silage. As no DiBP could be observed in the soil samples of these farms, the investigated silage was considered to be contaminated with DiBP due to migration from contact materials such as cling films, sails or sealants used during production, mixing or during storage at the farms (Cao, 2010; CDC, 2009). With respect to silage contamination with DEHP, both migration from contact materials and transfer from the environment might be important contamination pathways (Blüthgen, 2003; Cao, 2010; CDC, 2009; Cousins and Mackay, 2003; Staples et al., 1997). For DEHP, pasture was also observed to be a main contributor to the daily DEHP exposure in cows. For this type of feed, contamination can only be due to environmental transfer (Blüthgen, 2003; Cousins and Mackay, 2003; Staples et al., 1997). BBP and DEHP levels in manually obtained milk were lower than in mechanically obtained milk indicating that milk is contaminated with BBP and DEHP during the mechanical milking process as a result of migration from contact materials (Cao, 2010; CDC, 2009; Feng et al., 2005). The DEHP levels observed were in line with recent European studies on raw cow's milk (Cousins and Mackay, 2001; Sorensen, 2006) and were lower compared to older European (Castle et al., 1990; Sharman et al., 1994) and recent non-European studies (Feng et al., 2005; Kim et al., 2009). The declining trend of DEHP in European cow's milk over the last decades confirms the decreased use of DEHP in Europe due to the strengthening of the European legislation on the use of DEHP, and to the substitution of DEHP by other chemical substances (ECPI, 2010).

At industry level, phthalate contamination during the production process of milk powder was examined. In raw milk from the cooling tank of the factory, only DEHP was present in detectable amounts. After purification, separation, pasteurisation, standardisation and cooling, again only DEHP was found to be present, but in higher concentrations compared to the raw milk. DEHP levels increased even more after the second production stage (i.e. concentration, pasteurisation, homogenisation and spray drying). After this last stage, levels of DiBP and DnBP had also increased above the detection limit. After packing of milk powder in pouches and cans, an additional increase in levels of DEHP, DnBP, BBP and DiBP (only for cans) was observed. Based on these results, the conclusion may be made that DEHP, DiBP, DnBP and BBP migrated from contact materials into milk (powder) during production and packaging. Furthermore, this migration was most likely facilitated by heating (in the two pasteurisation steps) and by the large contact surface of milk powder (Castle, 2007).

Comparing phthalate concentrations in retail dairy products (i.e. milk, cheese and butter) with levels in raw cow's milk, revealed that, somewhere along the milk chain, milk and cheese were likely to be additionally contaminated with DiBP and DnBP. Since no milk or cheese factory was investigated in depth, specific contamination sources could not be determined for these types of products. Nevertheless, noteworthy to the retail milk product results was that, the same seasonal differences in phthalate concentrations were seen as observed at farm level. Furthermore, a positive relationship between fat content and DEHP content in the retail products was observed. This observation has also been reported by other research groups (Page and Lacroix, 1995; Sharman et al., 1994; Tsumura et al., 2002b).

The influence of home-cooking on the phthalate levels in foods was examined in a separate, more detailed study (see Chapter II.6). Understanding the impact of cooking on phthalate levels in food is important for several reasons. First of all, the food products that were considered in this study – i.e. starchy products (potato, pasta and rice), meat, fish and vegetables – are mainly eaten after cooking or baking. Thus, with respect to intake assessments, it is less relevant to determine phthalate levels in the raw, unprepared products. Furthermore, phthalates have been observed in coatings of cooking materials (Bradley et al., 2007) and may migrate from cookware into food during preparation. In the home-cooking study, the following processing methods were considered: boiling, steaming, frying, deep-frying and grilling. Processing reduced the number of positive samples for all phthalates except for DMP and DiBP, suggesting that home-cooking decreases the levels of most phthalates in food. In starchy products, levels of BBP and DEHP decreased significantly ($p<0.05$) after boiling. Although not significant, the same trend was observed for DEP, DiBP and DnBP. With respect to meat and fish, levels of DiBP and DEHP were always lower when the food products were fried in a frying pan with margarine than when they were fried in a non-stick frying pan without margarine. This might indicate that DiBP and DEHP migrated from the coating of the non-stick frying pan into the meat/fish product (Bradley et al., 2007).

IV.1.2 Modelling the occurrence of phthalates in foods

In this PhD project, the multimedia model "EN-forc" (ENvironmental Food transfer model for ORganic Contaminants) was developed in order to predict the occurrence of four phthalates (DEP, DnBP, BBP and DEHP) in foods and their resulting dietary exposure in Belgian adults. Modelling the occurrence of other phthalates was not possible due to a lack of sufficient input data (see also Section IV.4.2).

In a first step, the EN-forc model predicted the environmental transfer of DEP, DnBP, BBP and DEHP into a hundred basic agricultural products, such as leafy vegetables, root crops, tubers, meat, offal (liver and kidneys), milk and eggs starting from observed contaminant concentrations in air, surface water, sludge, manure, and so on (see Chapter III.1). In a second step, phthalate concentrations in 1,908 Belgian food products – both simple (e.g. butter) and complex (e.g. bread) – were predicted by making use of food recipes and by adding measured concentrations of about thirty non-agricultural (imported) food products to the model. In a final step, factors describing the impact of fat content, processing and packaging were applied to obtain predicted phthalate concentrations in packaged and/or processed Belgian food products (see Chapter III.2). After combining these predicted food concentrations with Belgian food consumption data (see also Section IV.1.3), the dietary exposure to phthalates in the Belgian population was assessed.

A general trend that could be observed for concentrations in plants, is that foliar crops were predicted to contain more phthalates than root crops and tubers. In fact, compared to observed concentrations, the occurrence of DEP, DnBP and DEHP in root crops and tubers was underestimated by the implemented plant models of EN-forc. Several reasons may explain these underpredictions. For example, compared to foliar crops, several contamination pathways (i.e. air gas phase exchange, wet and dry particle deposition and adherence of splashed soil particles) were not considered in the plant models for root crops and tubers. Mikes et al. (2009) for instance, revealed that polychlorinated biphenyls and organochlorine pesticides also enter radishes via air gas phase exchange, which may also be true for phthalates. Another reason may be that the input data used in the different modules of EN-forc may not be of sufficient quality. In various cases, EN-forc had to rely

on non-Belgian input data due to a lack of Belgian ones. For example, outdoor air gas phase concentrations were taken from the French study of Teil et al. (2006) to predict the transfer of phthalates via air gas phase exchange. So, until the working mechanisms of these dynamic plant models have been improved and/or better input data have been become available, it would be better to use measured phthalate concentrations in root crops and tubers instead of estimated ones in dietary intake assessments (Trapp and Schwartz, 2000).

Plant levels for DnBP, BBP and DEHP were already predicted in other studies, for example, using the EUSES model (Effting and van Veen, 1998; Müller et al., 2003). Compared to the EN-forc predictions and to measured concentrations in plants, the EUSES model predicts similar levels of DnBP and BBP in foliar crops and of BBP in root crops. However, EUSES calculations of DEHP in foliar crops underestimate the measured concentrations while predictions of BBP in root crops are more than two orders of magnitude higher compared to both measured and EN-forc concentrations.

With regard to animal products, eggs from free-range chickens were predicted to contain more phthalates than eggs from non-free-range chickens. This is most likely caused by the fact that EN-forc considers an additional exposure to phthalates through the intake of contaminated soil and pasture for free-range chickens. Compared to observed concentration levels in milk, EN-forc predicts better phthalate concentrations in cow's milk when using the equations of Rosenbaum et al. (2009), as opposed to the regression model of Travis and Arms (1988). With the exception of BBP in chicken meat, the same was true for the prediction of phthalate levels in different types of meat. As there are currently no suitable models available in literature for predicting the occurrence of phthalates in offal, the approaches of Rosenbaum et al. (2009) and Travis and Arms (1988) were also applied to liver and kidneys in EN-forc, although this is not their intended use. None of the two approaches proved to be adequate to calculate the biotransfer of phthalates to liver and kidneys. Model validation revealed that the EN-forc model predicts phthalate concentrations in animal products that are generally in line with measured concentrations and equally to more accurate than concentrations from other modelling studies, such as those from Effting and van Veen (1998) and from Müller et al. (2003).

By combining the predicted concentrations of phthalates in a hundred basic agricultural products, the measured concentrations of phthalates in about 30 non-agricultural (imported) food products and data regarding packaging, fat content and processing, EN-forc allows to predict phthalate concentrations in all food products listed in the FoodEx2 coding system of EFSA (2011a; 2011b). During the development of this module of the EN-forc model, a lot of assumptions had to be made. For instance, information about packaging distribution factors was lacking in the Belgian National Food Consumption Survey (Devriese et al., 2006) for several food groups. To obtain suitable data, information from other European surveys (Duffy et al., 2006a; 2006b; Poças et al., 2009) was used, but this was only possible assuming that the packaging distribution of food groups is comparable in other European countries.

Validation of the EN-forc model revealed that for the majority of the considered media, the EN-forc model is able to adequately predict phthalate levels in Belgian food products. Consequently, EN-forc may provide an inexpensive and rapid method to obtain food concentration estimates of chemicals that are hard to analyse or for which chemical analyses are expensive or time-consuming, such as phthalates. However, this will only be possible when sufficient high quality data are available to

specify the model inputs and after validation of the model for chemicals with a similar chemical and biological behaviour. For instance, the transfer of DiDP and DiNP – two high-production volume phthalates and known substitutes for DEHP – into food products could not be modelled by EN-forc due to a lack of sufficient and reliable physico-chemical property data and Belgian background concentrations. Taking this into account, it can be concluded that modelling and measuring should not be considered as independent approaches, but as complementary, synergistic approaches for obtaining concentrations of chemicals in food products.

IV.1.3 Estimating dietary phthalate exposure in the Belgian adult population

To estimate dietary exposure to phthalates in the Belgian adult population, two types of intake assessment approaches were used.

The first approach was applied within the PHTAL project (see Chapter II.3). In this project, the long-term dietary intake to DMP, DEP, DiBP, DnBP, BBP, DCHP, DEHP and DnOP was assessed by linking measured concentrations of phthalates in 572 foods with data from the most recent Belgian National Food Consumption Survey (Devriese et al., 2006). To link these two data sets, 90 food groups were defined, based on their nutritional composition and phthalate concentrations. Information about packaging was not considered during the linkage of these two data sets. Intake estimates were calculated probabilistically using the Monte Carlo Risk Assessment (MCRA) programme[7]. The intake of each phthalate was calculated for twelve different exposure scenarios, including the effect of home-cooking (2 scenarios), the effect of assigning concentrations to food samples with a phthalate concentration below the limit of quantification (lower, medium or upper bound; 2 x 3 = 6 scenarios) and the effect of replacing concentration ranges of a food group by the maximum phthalate concentration found in that food group (a worst case semi-probabilistic approach, 6 x 2 = 12 scenarios).

The second intake assessment approach was applied using the extended version of the EN-forc model (see Chapter III.2). In contrast to the first approach, this approach made use of predicted concentrations of phthalates in food. The contamination pathways considered by the EN-forc model were environmental transfer, migration from food contact materials (i.e. packaging), additional contamination from imported products and the effect of processing (i.e. washing, peeling, boiling, frying, and more). Predicted phthalate concentrations in 743 different food products – based on the FoodEx2 coding system of EFSA (2011a; 2011b) – were combined with data from the most recent Belgian National Food Consumption Survey (Devriese et al., 2006), analogous to the first approach. With the EN-forc model, only the long-term dietary intake of DEP, DnBP, BBP and DEHP in the Belgian adult population was estimated, since only for these four phthalates enough input data were at hand. This long-term intake was calculated semi-probabilistically using the “Mixtran” and “Distrib” algorithms from the American National Cancer Institute (Parsons et al., 2009; Tooze et al., 2010). The intake of each phthalate compound was calculated for two scenarios: one in which only predicted phthalate concentrations in food were used and another in which under- or overestimated concentrations were replaced by analysed concentrations.

[7] https://mcra.rivm/nl

In the PHTAL project, calculated intakes of DEHP were the highest, followed by DiBP and DnBP. Intakes predicted for the worst-case scenario (i.e. a semi-probabilistic approach using only maximum phthalate concentrations per food group) were five to ten times higher compared to the scenario that considered measured concentration ranges within a food group (i.e. a probabilistic approach). The impact of replacing concentrations below the limit of quantification on the assessed intakes was negligible for the phthalates with the highest intakes, i.e. DEHP, DnBP and DiBP, because the number of non-detects for these phthalates were rather low in the investigated food samples. Conversely, for phthalates like DMP and DnOP that were rarely present in foods (i.e. with a high number of non-detects), the effect of replacing values below the limit of quantification was rather high. Including the effect of home-cooking on phthalate concentrations resulted only in a negligible reduction of the assessed intakes, except for DiBP and DEHP. The results of the medium bound probabilistic scenario taking into consideration the effect of home-cooking were generally lower than or similar to intake estimates (also based on analysed concentrations) reported in international literature (COT, 2011; Dickson-Spillmann et al., 2009; Fromme et al., 2007b; MAFF UK, 1996; Petersen and Breindahl, 2000). The fact that the estimates calculated in this study were lower than estimates from other surveys may be due to the recent replacement of several phthalates (e.g. DEHP) by alternatives. This was for instance shown in a recent German study in which time trends in internal exposure to phthalates were investigated between 1998 and 2008 (Göen et al., 2011).

Dietary phthalate intakes predicted by the EN-forc model were the highest for DEHP, followed by DnBP, BBP and DEP. Replacing under- or overestimated EN-forc concentrations of phthalates in food by measured concentrations only significantly affected the intake distribution of BBP. Intake estimates were about two times higher when only predicted BBP concentrations were used, mainly due to an overestimation of BBP concentrations in meat by the EN-forc model. Of all considered subgroups of the Belgian adult population – i.e. total population, men, women, young adults, working population, elderly and very elderly – men and young adults were generally predicted to be exposed the most to DEP, DnBP, BBP and DEHP. A possible reason for these dissimilarities in intake estimates between gender and age categories is differences in dietary patterns (Vandevijvere et al., 2008). Considering that the use of phthalates can differ between countries (Wordsworth, 2007) and that some phthalates (e.g. DEHP) have been replaced by alternatives during the last two decades (ECPI, 2010), the predictions obtained in this study were in line with the results obtained in the modelling studies of Wormuth et al. (2006), Clark et al. (2011) and Heinemeyer et al. (2013). In contrast to this, predicted EN-forc results were higher than the results of Franco et al. (2007) using the EUSES model to calculate human exposure to DnBP and DEHP. As already stated by Franco et al. (2007), this dissimilarity was due to the fact that the EUSES model underestimates phthalate exposure.

Despite the fact that packaging was considered differently in the two intake assessment approaches, median and 95th percentile intake estimates of DEP, DnBP, BBP and DEHP in the Belgian adult population as predicted by the EN-forc model (considering packaging) agreed well with the results from the upper bound probabilistic scenario considering home-cooking (but neglecting packaging) conducted within the PHTAL project. This indicates that the contribution of packaging materials to the contamination of food products with phthalates and thus to human dietary exposure to phthalates may not as important as originally thought. Moreover, this means that phthalate contamination in the Belgian food chain mainly derives from other sources than packaging such as

the environment or food contact materials used during cultivation or production. This observation confirms the conclusions that were made during the PHTAL project when contamination pathways for phthalates in the Belgian food chain were investigated (see Chapter II.2).

Not only high-fat foods (e.g. meat and meat products), but also low-fat food products like fruits, bread and vegetables were observed to be important contributors to dietary phthalate intake in Belgian adults. The same observation was more or less made in a recent British survey (COT, 2011). Consequently, consumers who try to make conscious food choices are also exposed to phthalates through their diet. As a matter of fact, several studies indicated that conscious consumers are more exposed to phthalates than others (Dickson-Spillmann et al., 2009; Sathyanarayana et al., 2013). This means that, even if people are aware of the occurrence of chemicals in foods and/or try to make conscious food choices, dietary exposure to phthalates cannot be avoided.

Although the food consumption survey data used in this PhD were based on the most recent representative data set for the Belgian adult population (Devriese et al., 2006), it was still collected ten years ago. It is very likely that consumption patterns have changed over the last ten years due to the presence of new food products on the market. In this PhD dissertation, it was assumed that the food consumption pattern of Belgian adults did not change substantially since 2004 for the food groups contributing most to the dietary exposure to phthalates. Furthermore, it is possible that food consumption data collected in national surveys are under- or overreported by the study participants. Such biases are hard to overcome and certainly will have an effect on the results of the intake assessment on the individual level. At the population level, however, it can be assumed that such data give reliable estimates.

For the intake assessment approach of the PHTAL project, measured concentrations of the eight considered phthalates in 572 different food samples were used. This number of samples is extensive, especially considering the fact that the analysis of phthalates in foods is laborious work due to the complex methodology of sample handling and analysis, constantly avoiding external contamination (David et al., 2003). However, compared to the total number of food items present on the Belgian market possibly containing phthalates, and considering the numerous brands and packaging materials, this number of analysed food samples is still low. A rather inexpensive and fast solution to meet such analytical problems is to make use of model approaches to predict phthalate concentrations in foods and their resulted dietary exposure in humans. In this PhD dissertation, the EN-forc model proved to be a good alternative to predict the long-term daily dietary exposure of DEP, DnBP, BBP and DEHP in the Belgian adult population in a semi-probabilistic way. By extension, this model might also be a useful dietary exposure tool for other organic chemicals that are hard to analyse.

IV.2 Relevance to public health

During the last two decades, several food incidents have been reported in the Belgian media and debated by the general public: the dioxin crises of 1999 and 2003, the contamination of Chinese milk with melamine in 2008, food products polluted with methylbenzophenone as a result of packaging migration in 2009, the presence of the enterohaemorrhagic *Escherichia coli* (EHEC) bacterium in several types of vegetables in 2011 and more recently, the horsemeat scandal in 2013 (FASFC, 2014a). Concerning phthalates specifically, the Food and Drug Administration of Taiwan reported in 2011 that the phthalates DEHP, DnBP and DiNP were illegally added to clouding agents in several Taiwanese foods and beverages (Yang et al., 2013). These and other incidents have led to a high level of food safety concern among the general public and have resulted in food safety being a top priority for the Belgian Federal Agency for the Safety of the Food Chain (FASFC), by laying down, implementing and enforcing food safety measures.

This chapter deals with the relevance of this PhD project to public health. First, the contribution of the diet to the integral exposure of the eight phthalate compounds considered in this study is discussed. Then, the availability of exposure limit values for phthalates is described. Subsequently, dietary exposure estimates for phthalates are evaluated for different subgroups of the Belgian population and potential health effects associated with integral phthalate exposure are described. The chapter ends by explaining how policy makers have dealt with data gaps and scientific uncertainty during the evaluation of the risks of chemical substances (including phthalates) for public health.

IV.2.1 Contribution of the diet to integral phthalate exposure

As elaborated in Section I.4.1, people can be exposed to phthalates via four types of routes: (1) oral ingestion (of food and drinks, dust, soil, enteric-coated tablets and by mouthing objects), (2) inhalation (of indoor air, outdoor air and sprays), (3) dermal absorption (of phthalate containing consumer products such as personal care products) and (4) parenteral exposure from medical devices. Depending on the phthalate compound and the population group considered, the contribution of every of these exposure routes to integral phthalate exposure is different.

Several research groups have investigated to contribution of the above mentioned exposure routes to integral phthalate exposure (Clark et al., 2011; Lee et al., 2014; Müller et al., 2003; Wormuth et al., 2006). Table 50 summarises the estimated contribution percentages of food ingestion to integral phthalate exposure in these studies, focusing on the phthalated compounds considered in this PhD project. With the exception of dietary DEP exposure in the Canadian population aged above 0.5 years, the role of the diet in relation to integral DMP and DEP exposure was observed to be negligible. Contribution percentages of food ingestion varied between 0 and 10% for DMP and between 3 and 10% (and 54-60% for Canadians above 0.5 years old) for DEP. In all age categories, integral DiBP exposure seemed to be mainly caused by the ingestion of contaminated food: contribution percentages for DiBP via food ingestion amounted up to 60% for children below the age of four and up to 85-95% for the older age categories. In corresponding age classes, the contribution of food ingestion to integral DnBP exposure seemed to be higher in European than in non-European countries. For instance, in Canadian infants, food was responsible for 13-46% of the total exposure to DnBP whereas in Danish infants, DnBP exposure was for almost 100% caused by the ingestion of

contaminated food products. With respect to BBP, it can be concluded that food contribution percentages vary considerably amoung the conducted studies: ranges between <6 and 98% have been reported. At last, for DEHP, food ingestion can be seen as a major contributor to the integral exposure, as was the case for DiBP. Estimated DEHP food contribution percentages were mostly higher than 75%.

Table 50: Contribution of food ingestion (in %) to integral phthalate exposure in several populations.

Population	Country/ Continent	DMP	DEP	DiBP	DnBP	BBP	DEHP	Reference
Infants (0-0.5 y.)	Canada	<5	7	-	13-46	<6	34-76	Clark et al., 2011
Infants (0.5-1 y.)	Denmark	-	-	-	99	98	96	Müller et al., 2003
Infants-toddlers (0-4 y.)	Europe	3	3	60	60-70	20	50	Wormuth et al., 2006
Toddlers (2 y.)	Korea	-	-	-	25-41	26-50	63-75	Lee et al., 2014
Toddlers (2 y.)	Denmark	-	-	-	50	49	54	Lee et al., 2014
Toddlers (1-6 y.)	Denmark	-	-	-	99	98	88	Müller et al., 2003
Children (4-10 y.)	Europe	3	5	85	60	73	90	Wormuth et al., 2006
Children (7-14 y.)	Denmark	-	-	-	95-99	96	82	Müller et al., 2003
Teenagers (11-18 y.)	Europe	0	3	95	40-60	20	95	Wormuth et al., 2006
Toddlers-adults (0.5-70 y.)	Canada	<5	54-60	-	75	68-77	95	Clark et al., 2011
Adults (15-80 y.)	Denmark	-	-	-	91-99	97	76	Müller et al., 2003
Adults (18-80 y.)	Europe	10	10	95	80-90	60	98	Wormuth et al., 2006
RANGE (%)		0-10	3-60	60-95	13-99	<6-98	34-98	

Based on the studies summarised in Table 50, it can be concluded that food is a major exposure route for the phthalates DiBP, DEHP and to some extent also for DnBP and BBP (a wide range of contribution percentages was found) whereas this exposure route is rather negligible for phthalates like DMP and – with the exception of Canadians above 0.5 years old – also DEP. With regard to DCHP and DnOP – the two other phthalates considered in this PhD project – no studies could regrettably be found that investigated the role of the diet in relation to integral exposure. So, for these two substances, it is still unclear wether food ingestion contributes substantially to human exposure to phthalates.

IV.2.2 Availability of exposure limit values for phthalates

Four of the eight phthalates considered in this thesis have received a TDI value by EFSA (2005a; 2005b; 2005c; WHO, 2003) or WHO (2003), namely DEP, DnBP, BBP and DEHP. Simultaneously, US EPA (2007) has established oral RfD values for these compounds. In Table 51, an overview is given of these exposure limit values as well as the end-points observed in animals, NOAELs and uncertainty factors that were used to derive them. As can be noticed, TDI and RfD values differ from each other since they are extrapolated from other NOAEL/LOAEL values obtained in toxicological studies and since other uncertainty factors were taken into account to extrapolate the data of the animal studies to human health. Moreover, the corresponding TDI and RfD values of DEP, DnBP, BBP and DEHP are based on other observed end-points in animals: while the current TDIs are based on developmental and/or reproductive effects observed in (multi)generation studies of mice or rats, the RfDs are based on altered growth and consumption rates, modified organ weights or increased mortality observed in adult rats or guinea pigs. Although not considered in this dissertation, but worth mentioning is that EFSA (2005d; 2005e) has also set-up TDI values for the phthalates DiNP and DiDP. The values of these TDIs as well as the information used to establish them are summarised in Table 51 as well.

EFSA (2005f) also investigated whether a group TDI could be established for DnBP, BBP, DEHP, DiNP and DiDP. Such a group TDI can only be employed if 1) exposure to several members of a structurally related series of chemicals is likely to occur frequently and 2) several members of the series have been demonstrated to have a common target organ(s) cellular target(s) and the same mode of action. Although DnBP, BBP and DEHP act on the same target organs (i.e. the reproductive organs), their profile on effects at the hormonal and cellular level are not identical. Moreover, the two remaining phthalates – DiDP and DiNP – primarily affect the liver rather than the reproductive organs. Consequently, EFSA (2005f) concluded that a group TDI for these five phthalates could not be allocated.

Table 51: TDI and RfD values of different phthalate compounds including the information (end-points observed in animals, NOAELs and uncertainty factors) used to establish these exposure limit values.

Compound	Limit value (mg/kg bw/day)	End-points observed in animals	NOAEL (mg/kg bw/day)	Uncertainty factor	Reference
Tolerable Daily Intake					
DEP	5	Developmental effects (decreased fetal weight) in mice (offspring)	1600	300	WHO (2003)
DnBP	0.01	Developmental effects (germ cell development) in rats (offspring)	2 [a]	200	EFSA (2005a)
BBP	0.5	Developmental effects (decreased AGD) in rats (multigeneration study)	50	100	EFSA (2005b)
DEHP	0.05	Testicular (decreased testis weight) and developmental (germ cell depletion) effects in rats (multigeneration study)	5	100	EFSA (2005c)
DiNP	0.15	Hepatic (spongiosis hepatis, increased levels of liver enzymes and increased liver weight) and renal (increased kidney weights) effects in rats (two-year chronic toxicity study)	15	100	EFSA (2005d)
DiDP	0.15	Hepatic effects (microscopic lesions) in dogs (13-week oral study) and developmental effects (decreased F2 offspring survival) in rats (multigeneration study)	15	100	EFSA (2005e)
Reference Dose for chronic oral exposure					
DEP	0.8	Decreased growth rate, food consumption and altered organ weights in adults rats (subchronic study)	750	1000	US EPA (2007)
DnBP	0.1	Increased mortality in adult rats (subchronic to chronic study)	125	1000	US EPA (2007)
BBP	0.2	Increased liver-to-body weight and liver-to-brain weight ratios in adult rats (six-month study)	159	1000	US EPA (2007)
DEHP	0.02	Increased relative liver weight in adult guinea pigs (subchronic to chronic study)	19 [a]	1000	US EPA (2007)

[a] LOAEL instead of NOAEL.

To correctly assess the risks related to phthalate exposure (and other chemicals), it is essential that exposure limit values used for the risk assessment are based on end-points observed in animals that are relevant and of most importance to the toxicity of humans. The phthalates DEP, DnBP, BBP and DEHP are all classified as Category 1 substances on the European priority list of chemicals with potential endocrine disrupting activities (European Commission, 2014b). Hormones play an important role in the correct development and functioning of many systems in the human body such

as the reproductive system, brains and the neuro-endocrine system. As a consequence, exposure to compounds such as DEP, DnBP, BBP and DEHP is the most critical during those periods of human life, in which the endocrine system is undergoing changes, namely, during pregnancy (embryonal and fetal phases), infancy (including breast-feeding period), adolescence and during senescence (Superior Health Council, 2013; WHO, 2002). These findings indicate that the exposure limit values of DEP, DnBP, BBP and DEHP are preferably based on developmental effects observed during (multi)generation studies in animals. This is the case for the current established TDI values of these four phthalates, but not for the corresponding RfD values.

Although not classified as Category 1 substances, DiBP, DiNP, DiDP and DnOP are also phthalates that are related to endocrine disrupting activity, i.e. they are classified as Category 2 (DiBP, DiNP and DiDP) and Category 3 (DnOP) substances on the European priority list (European Commission, 2014b). This means that for these phthalate compounds, there is currently only some *in vitro* evidence (Category 2) or even no evidence or data available regarding the endocrine disrupting activity (Category 3) of these substances. At the moment, only DiDP and DiNP have received a TDI value by EFSA (2005d; 2005e); for DiDP and DnOP, exposure limit values are lacking. Furthermore, the TDIs of these two compounds (especially DiNP) are mainly based on hepatic and/or renal effects observed in adult animals and not on reproductive or developmental effects in (multi)generation studies (EFSA, 2005d; 2005e). When more information regarding the endocrine disrupting activity of DiBP, DiNP, DiDP and DnOP becomes available, it will be desirable that authorities like EFSA, WHO and US EPA consider establishing or re-evaluating exposure limit values for these phthalates.

Besides, studies have also indicated that the toxicological effects of several endocrine disrupting compounds (including some phthalate diesters and monoesters like DEHP, MnBP and MBzP) are not having a “classic” (linear) monotonic dose-response relationship (an overview of these studies can be found in the review of Vandenberg et al., 2012). Moreover, people are also not only exposed to single chemical substances, but to mixtures of chemical compounds with endocrine disrupting activity. As a result, synergistic, antagonistic or additive effects may be observed (Grandjean et al., 2006; Kortenkamp, 2008). As also expressed by others (Grandjean et al., 2006; Superior Health Council, 2013; Vandenberg et al., 2012), this makes the evaluation of the overall health risks of exposure to endocrine disrupting compounds such as phthalates very complex. More research in order to clarify the toxicological effects of these compounds (single substances as well as mixtures) is urgently needed. However, such research is very expensive. Scialli (2008) for instance reported that – in order to meet the REACH data requirements regarding reproductive and developmental toxicity – the costs of performing multigeneration studies with rats are up to several hundred thousand euros per chemical substance registered at the ≥ 10,000 tonnes/year band. Consequently, it is almost self-evident that manufacturers are only willing to perform those toxicity tests that are strictly necessary in order to fulfil the legal requirements. Transparency and knowledge sharing, i.e. the collaboration of manufacturers with scientists and public authorities, is one of the only things that could be done in order to learn more about the implications of exposure to compounds such as phthalates to human health (COMEST, 2005).

IV.2.3 Dietary phthalate exposure in the Belgian population

In this thesis, the dietary exposure to phthalates was calculated for the Belgian adult population. Two estimation approaches were considered: an intake estimation based on measured phthalate levels in foods (Chapter II.3) and one based on modelled phthalate concentrations in foods (Chapter III.2). In both approaches, the intake estimates of DEP, DnBP, BBP and DEHP were compared with their corresponding TDI values (Table 52). Depending on the considered scenario, dietary exposure to DEP, DnBP, BBP and DEHP in Belgian adults represented <0.1, 1-12, <0.1-1 and 3-28% of the corresponding TDI values, respectively (calculated from P50 and P95 intakes). Thus, the assessed exposures expressed as a percentage of the TDI obtained in this PhD study (Table 52) were all much lower than the observed contribution percentages of the diet to integral exposure (Table 50).

Table 52: Estimated dietary phthalate intakes for several subgroups of the Belgian population (in µg/kg bw/day). The values between parentheses show the estimated intakes in relation to their respective TDI values (in %).

Population (Reference)	Scenario		Dietary intake in µg/kg bw/day (% of TDI)			
			DEP	DnBP	BBP	DEHP
Infants (FASFC, 2014b)	Deterministic, average concentrations (medium bound), P95 consumption rates	-	-	-	-	15 (30%)
	Deterministic, P90 concentrations (medium bound), P95 consumption rates	-	-	-	-	27 (54%)
Preschoolers (Sioen et al., 2012)	Probabilistic, considering preparation, medium bound (MCRA)	P50	0.11 (<0.1%)	0.20 (2%)	0.12 (<0.1%)	3.5 (7%)
		P95	0.17 (<0.1%)	0.30 (3%)	0.21 (<0.1%)	5.4 (11%)
	Worst case, considering preparation, medium bound (MCRA)	P50	0.44 (<0.1%)	1.3 (13%)	0.53 (1%)	18 (36%)
		P95	0.75 (<0.1%)	2.3 (23%)	1.0 (2%)	29 (58%)
Adults (Chapter II.3)	Probabilistic, considering preparation, medium bound measured concentrations	P50	0.04 (<0.1%)	0.08 (1%)	0.05 (<0.1%)	1.5 (3%)
		P95	0.08 (<0.1%)	0.16 (2%)	0.12 (<0.1%)	2.9 (6%)
	Worst case, considering preparation, medium bound measured concentrations	P50	0.16 (<0.1%)	0.57 (6%)	0.24 (<0.1%)	7.2 (14%)
		P95	0.34 (<0.1%)	1.2 (12%)	0.53 (1%)	14 (28%)
Adults (Chapter III.2)	Semi-probabilistic, considering packaging and preparation, modelled concentrations	P50	0.04 (<0.1%)	0.15 (2%)	0.08 (<0.1%)	1.4 (3%)
		P95	0.06 (<0.1%)	0.27 (3%)	0.16 (<0.1%)	2.4 (5%)
Adults (FASFC, 2014b)	Deterministic, average concentrations (medium bound), P95 consumption rates	-	-	-	-	1.2 (2%)
	Deterministic, P90 concentrations (medium bound), P95 consumption rates	-	-	-	-	2.1 (4%)

Although not reported in Chapter II.3 of this dissertation, but worth mentioning, is that in the PHTAL project, dietary exposure to phthalates was also estimated for Belgian preschool children (Sioen et al., 2012). Just as for Belgian adults, no exceedance of the TDI was to be expected for the dietary intake of DEP, DnBP and BBP for Belgian preschoolers as calculated TDI contribution percentages of dietary exposure to these phthalates in Belgian preschoolers (Table 52) were far below the contribution percentages of the diet to integral exposure observed in European studies (Table 50). However, this did not hold true for DEHP. In the worst case scenario, the 95th percentile of the dietary DEHP intake in Belgian preschool children represented 58% of the TDI. This percentage is within the range of the observed contribution percentages of the diet to integral DEHP exposure in European children (i.e. 50-90%; see also Table 50). This means that there is a risk that, in some cases, the TDI value of DEHP would be exceeded for Belgian preschoolers.

Recently, the Scientific Committee of the FASFC (2014b) evaluated the risk of the daily (chronic) exposure to DEHP based on the results of the FASFC monitoring program between 2008 and 2012. In this monitoring program, DEHP was analysed in 130 baby food products and 161 general food samples packaged in glass jars, since DEHP is often present in the metal lid coatings of glass jars. For adults, exposure to DEHP seemed to imply no significant health risk, even for the most pessimistic scenario where high consumption (i.e. P95 values) and high food contamination levels (i.e. P90 (medium bound) concentrations) were assumed. The contribution of the estimated dietary DEHP intakes to the corresponding TDI value amounted to 2-4% (Table 52). Since ingestion of contaminanted food is the major exposure route of DEHP accounting for a contribution to the exposure of 34-98% (Table 50), it is unlikely that the integral exposure would reach the TDI. For infants (<1 year), dietary exposure to DEHP also remained below the TDI, i.e. intake estimates of 30 and 54% of the TDI were calculated for a normal (average concentrations) and worst-case scenario (P90 concentrations), respectively (Table 52). However, the Scientific Committee of the FASFC (2014b) highlighted that, in addition to contamination of food via food contact materials, also other sources of contamination (e.g. the environment) and other sources of exposure (e.g. plastic toys and dust) to DEHP are possible (see also Table 50). Consequently, when considering the combined exposure from all possible contamination pathways, it is not unlikely that for Belgian infants, the TDI value would be exceeded in some situations.

In conclusion, it can be stated that no TDI exceedances are to be expected with regard to DEP, DnBP, BBP and DEHP exposure for Belgian adults. However, for the younger age categories (i.e. Belgian infants and preschool children), there might be a chance that – in specific situations – the TDI of DEHP will be exceeded. Furthermore, it should be noticed that for the other four phthalates studied in this PhD dissertation – namely DMP, DiBP, DCHP and DnOP – no risk assessment could be done due to a lack of available exposure limit values. So, for these compounds, it is still unclear if health risks related to phthalate exposure are to be expected.

IV.2.4 Health effects associated with integral phthalate exposure in the Belgian population

Within the framework of this PhD project, it is relevant to describe urinary phthalate metabolite concentrations available for some Belgian subpopulations (Table 4) and compare them with urinary levels from studies in which phthalate exposure was related to potential adverse health effects.

In American adult men, relationships have been found between DnBP and BBP exposure and altered hormone (FSH and inhibin B) levels in serum (Duty et al., 2005b) and between DEHP exposure and DNA damage in sperm (Hauser et al., 2007). In these American studies, median (P95) single-spot urinary metabolite concentrations amounted to 14 (75) µg/l for MnBP, 6.9 (37) µg/l for MBzP and 7.7 (112) µg/l for MEHP. By way of comparison, median (P95) first morning urinary levels of these phthalate metabolites in Flemish adolescents were 39 (116) µg/l for MnBP, 30 (163) µg/l for MBzP and 3.7 (18) µg/l for MEHP (Geens et al., 2014). As can be noticed, MnBP and MBzP levels were higher in Flemish urine samples than in the American ones whereas to opposite can be observed for MEHP. However, this observation should be taken with caution since the subpopulations (American adults *vs.* Flemish adolescents) and types of urine samples (single-spot *vs.* first morning) investigated are not fully comparable to each other.

By measuring urinary phthalate metabolite concentrations in American pregnant women, Swan et al. (2008; 2010; 2005) associated prenatal exposure to DEP, DnBP, DiBP, BBP and DEHP with negative health outcomes in male children like a shortened AGD and/or reduced masculine play behaviour. The single-spot urinary phthalate metabolite concentrations observed in these American women were quite similar to the phthalate levels detected in the first morning urine samples of Belgian mothers during the DEMOCOPHES project (The Belgian Steering Committee on HBM, 2013). For instance, in the study of 2008, Swan et al. observed median urinary MEP, MnBP and MEHP concentrations of 277, 26 and 6.2 µg/l, respectively, in women of which their son had a significant shorter AGD. In Belgian mothers, median (P95) concentrations amounted to 34 (240), 31 (119) and 2.3 (9.1) µg/l, respectively. For the study of Swan et al. conducted in 2005, Marsee et al. (2006) converted the measured urinary phthalate metabolite levels of the pregnant women to daily internal phthalate diester exposure values. By using the model of David (David, 2000; see also Equation 1 in Section I.6.1.1), Marsee et al. (2006) calculated median (P95) exposure values of 5.3 (90) µg/kg bw/day for DEP, 0.7 (1.9) µg/kg bw/day for DnBP, 0.1 (0.3) µg/kg bw/day for DiBP, 0.4 (1.7) µg/kg bw/day for BBP and 1.3-2.4 (9-17) µg/kg bw/day for DEHP. Although all these obtained values were substantially lower than the current exposure limit values (Table 51), Swan et al. (2005) found that in male children negative health outcomes were associated with the measured phthalate exposure levels. As already indicated in Section IV.2.2, this may be the consequence of the fact that people are simultaneously exposed to several endocrine disrupting compounds that may potentially interact.

Both comparisons reveal that the urinary phthalate metabolite concentrations available for Belgian subpopulations are similar to or even higher than levels of studies in which phthalate exposure was linked to several adverse health effects. Although these findings are based on epidemiological study results – meaning that the observed health outcomes are not necessarily causally linked to phthalate exposure – this might indicate that the health of the Belgian population is negatively affected by the simultaneous exposure to phthalates and other endocrine disrupting compounds.

IV.2.5 Data gaps and scientific uncertainty: the precautionary principle

The precautionary principle aims at ensuring a higher level of protection through preventative decision-taking in the case of risk. This principle is especially used by policy makers in situations where the scientific data available do not permit a complete risk evaluation of possible dangers to human, animal or plant health or to the environment. An approach based on the precautionary principle may only be implemented in situations where the three preliminary condictions are met, namely (1) potentially adverse health effects are identified, (2) the scientific data available are evaluated as complete as possible and where possible, (3) the degree of scientific uncertainty is identified at each stage. The dimension of the precautionary principle goes beyond problems associated with short or mid-term risk approaches; it also concerns long term risks and the well-being of future generations (COM, 2000; COMEST, 2005).

Plenty of the current policy debates about endocrine disrupting compounds have been concentrated on the precautionary principle, since there is still a lot of uncertainty regarding the potential adverse effects of these substances on the human endocrine system. Bisphenol A for instance, has received much attention during the last years due to its reproductive and developmental effects observed in low dose (i.e. in the µg/kg bw range) animal toxicity studies (Tyl, 2014). Particular concern was expressed with regard to the use of bisphenol A in polycarbonate baby bottles. Based on the

information available, Health Canada (2010) suggested to apply the general principle of ALARA – as low as reasonably achievable – to limit the exposure to bisphenol A from food packaging applications of newborns and infants. In Europe, Commission Directive 2011/8/EU prohibits from 1 March 2011 the manufacture and from 1 June 2011 the import and sale of bisphenol A containing plastic infant feeding bottles (Official Journal of the European Union, 2011c). This Directive was the consequence of a preventive measure taken by the European Commission on the basis of the precautionary principle.

The precautionary principle has also been applied by the European Commision to regulate the use of phthalates in toys and childcare articles (see also Section I.5.3). In 1999, the Commission made a first proposal in order to introduce a ban on the use of six phthalates (i.e. DnBP, BBP, DEHP, DnOP, DiNP and DiDP) in toys and childcare articles for oral application in children under the age of three. In the discussions that followed on this proposal, the decision was made to wait until new scientific data would become available from a series of comprehensive risk assessments that had been initiated in the meantime. By considering this newly gathered information among others, the new proposal aimed to take necessary measures – in line with the precautionary principle – while making a distinction between the different phthalate compounds regarding their individual potential to cause risks to children. The phthalates DEHP, DnBP and BBP turned out to be toxic for reproduction and were accordingly classified as CMR substances (category 2). Based on several considerations (e.g. exposure from toys is possibly the most important source that can be controlled by concrete measures), the Commission decided that a total ban of these substances from products intended for children such as toys was justified. With respect to DiNP, DiDP and DnOP, the Commission concluded that scientific information was lacking or conflictual, but that it could not be excluded that these substances pose a potential risk when used in toys and childcare articles. Restrictions on the use of these three compounds in toys and childcare articles should be introduced, but the restrictions should be less severe than the ones proposed for DnBP, BBP and DEHP. Therefore, the Commission decided to ban the use of DiNP, DiDP and DnOP in toys and childcare articles that can be placed in the mouth by children under the age of three (COM, 2005; Official Journal of the European Union, 2005).

IV.3 Suggestions for policy development

IV.3.1 Suggestions for legislation about food products

Both the monitoring (Chapter II.3) and modelling (Chapter III.2) approaches carried out in this PhD dissertation to estimate dietary exposure, revealed that grains, meat, fruit, milk and derived products contributed most to the dietary exposure to the eight and four considered phthalate compounds in the Belgian adult population, respectively. Since grains, meat, fruit, milk and derived products are consumed frequently and usually in high amounts, it is important to continue the efforts by authorities to reduce the presence of phthalates in these types of foods. One possibility is to set maximum residue levels (MRLs) for certain phthalates in certain foods, e.g. for DiBP, DnBP and DEHP in grain and grain-based products (such as bread). Such an approach has already been established for several mycotoxins in Regulation No 1881/2006 of the European Commission (Official Journal of the European Union, 2006a). For setting up MRLs, authorities may make use of modelling approaches such as EN-forc, since these allow to evaluate the potential impact of the presence of a chemical in a particular food product on food safety and public health, given a specific MRL level.

IV.3.2 Suggestions for legislation about food contact materials

The monitoring campaigns in which phthalate levels were analysed in packaged food items and in packaging materials (Chapters II.1, II.2, II.5 and II.6) showed that phthalates were present in almost every foodstuff present on the Belgian food market, irrespective of the packaging type of the investigated foods. This may indicate that the examined food items were already contaminated with phthalates before packaging and thus were contaminated as a result of environmental transfer and/or due to the use of phthalate containing contact materials used during cultivation, production or transport (Dickson-Spillmann et al., 2009; Nehring, 2006). Nevertheless, foods from the monitoring campaigns that were packaged in paper/cardboard generally contained more DiBP than similar unpackaged or differently packaged food products. The same trend was observed for BBP and DEHP in canned food products. Both observations were mostly caused by the use of DiBP, BBP and/or DEHP in printing inks, coatings and adhesives in the packaging materials (BfR, 2007; Bradley and Castle, 2007; Castle et al., 1989; Nerin et al., 1993).

Although only investigated qualitatively in the PHTAL project and thus not reported in this thesis, gaskets of metal closures for glass jars and crown caps for glass bottles were also shown to contain DiBP, DnBP, BBP and DEHP. The latter phthalate compound has also been found in gaskets of metal closures in a Swiss market survey (Fankhauser-Noti et al., 2006).

In contrast to plastic food contact materials, for which specific migration limits and phthalate usage restrictions (see Section I.5.6) are imposed by Regulation 10/2011 (Official Journal of the European Union, 2011b), there currently does not exist any legislation at European level specifying explicit migration limits or restrictions for the use of phthalates in printing inks, paper, and so on (Gartner et al., 2009). Based on the results obtained in this PhD thesis and from other monitoring and exposure estimation studies published in literature, policy makers might consider imposing specific migration limits for this type of applications. Another possibility for authorities to protect food products against phthalate contamination would be the implementation of maximum permitted levels for the use of phthalates in food contact materials, as has been done for the use of DEHP, DnBP, BBP, DiNP, DiDP and DnOP in toys and childcare articles (Official Journal of the European Union, 2005; 2006b).

Besides the direct use of phthalates in food contact materials, some studies have also indicated that (traces of) phthalates can be present in recycled (packaging) materials, in particular materials of paper or cardboard (e.g., BfR, 2007; Bradley, 2012; Gartner et al., 2009). This might also be something to take into consideration by the European Commission when developing guidelines for the (safe) use of recycled materials intended for food purposes.

IV.3.3 Possible alternative substances to phthalates

As explained in Section I.5.1, the authorisation procedure of the REACH regulation aims to assure that substances of very high concern, such as the phthalates DEHP, DiBP, DnBP and BBP, are progressively replaced by suitable alternatives without jeopardising the good functioning of the European internal market (ECHA, 2014). This is a huge challenge for both policy makers and manufacturers as alternative substances should be less harmful to human health and should, at the same time, be technically able to replace the original substances in all user applications. Moreover, especially for manufacturers, the cost of using these alternatives should preferably not be higher compared to the original.

In 2010, the Danish Environmental Protection Agency investigated which plasticisers could be used as alternatives to the phthalates DEHP, DnBP and BBP (Danish EPA, 2010). This study was mainly based on technical descriptions of alternatives to phthalates from manufacturers' assessments of relevant uses and market experiences, especially from the use in toys, food packaging materials and medicinal products. A detailed assessment was carried out for ten possible non-phthalate alternative substances: alkylsulphonic phenylester (ASE), acetyl tri-*n*-butyl citrate (ATBC), a mixture of 12-acetoxy-stearic acid, 2,3-bis(acetoxy)propyl ester and octadecanoic acid, 2,3-bis(acetoxy)propyl ester (COMGHA), diethylene glycol dibenzoate (DEGD), dipropylene glycol dibenzoate (DGD), di(2-ethylhexyl)terephthalate (DEHT), diisononyl adipate (DINA), di-isononyl-cyclohexane-1,2-dicarboxylate (DINCH), glycerol triacetate (GTA) and trimethyl pentanyl diisobutyrate (TXIB). Based on several animal studies, the Danish EPA (2010) concluded that all alternative non-phthalate substances assessed are expected to demonstrate low acute toxicity. For three of the alternatives, data showed that they are not carcinogenic, mutagenic or harmful to reproductive capacity; for the other alternatives, data were lacking for at least one critical parameter. Although not statistically significant, the toxicological data for DEGD and DGD suggested that these substances may have an effect on reproductive capacity. With respect to environmental properties, none of the investigated substances met the criteria for being persistent, bioaccumulative and toxic (PBT) in the aquatic environment or being very persistent and very bioaccumulative (vPvB), although – with the exception of GTA – all substances showed one or two of these properties.

Whether it is technically possible to use these alternative substances to replace DEHP, DnBP, BBP and DiBP is described in a Danish proposal for a restriction on these four phthalates (Danish EPA, 2011). In some cases, a mixture of different alternatives has to be used in order to achieve the same technical results in the product. However, this equally holds true for the original phthalate compounds. No single alternative substance can replace the four phthalates for all applications in which they are used today. Moreover, the alternatives have different properties that prevent them from being optimal under all circumstances. Therefore, it is recommended that enterprises first carefully review alternative substances to DEHP, DnBP, DiBP and BBP in the light of their specific application and purpose.

To date, DEHP has mostly been replaced by two other phthalate compounds, namely DiNP and DiDP (ECPI, 2010). These two phthalates are classified as Category 2 substances on the European priority list of chemicals with potential endocrine disrupting activities, which means that they are suspected to be less endocrine disrupting than DEHP, which is classified as Category 1 substance (European Commission, 2014b). DiNP and DiDP are often produced by the same companies that produce DEHP and exhibit physico-chemical properties very similar to those of DEHP. The price of production of these two phthalates is slightly higher (i.e. about 10% more) compared to DEHP. Alternative non-phthalate plasticisers found to replace DEHP on the market are DINA, DINCH, DEHT, ATBC and ASE, among others. The cost of these alternatives varies from slightly higher (i.e. less than 10% more) to significantly higher (up to three times more) compared to DEHP. Alternative substances to replace DnBP and BBP were DEGD and DGD among others. In contrast to the non-phthalate alternatives for DEHP, the costs of these alternatives are very similar to the prices of DnBP and BBP (Danish EPA, 2010).

The potential cost of replacing DEHP, DnBP, BBP and DiBP by other phthalates (e.g. DiNP and DiDP) as well as by non-phthalate alternatives (e.g. ATBC and TXIB) within the European market has been calculated by the European Chemicals Agency (ECHA, 2013). In their assessment, ECHA only focused on substance substitution and thus did not consider other measures for reducing the emissions of the substances, such as pollution control during manufacturing. ECHA concluded that extensive experience exists in replacing DEHP and BBP with alternative phthalates and that uncertainties on the costs of substitution are mainly due to possible price differences between alternative phthalates and DEHP and BBP. For DnBP and DiBP, the possibilities for substitution are more diverse with a wider range of alternatives, but with similar prices to the existing substances. Using least-cost alternatives (i.e. a replacement by principally other phthalate compounds), the costs of replacing DEHP and BBP were around €200/tonne and around €300/tonne for replacing DnBP and DiBP within the European market.

IV.4 Recommendations for future research

IV.4.1 Analytical recommendations

During the PHTAL project, eight phthalate compounds were analysed in three different food matrices (low-fat foods, high-fat foods and aqueous-based beverages) and in several packaging types (cardboard, metal, plastic, and so on; see Chapter II.1). Unfortunately, method validation revealed that the GC-EI-MS chromatograms of some protein-rich food products (mainly beer samples) were perturbed. In these samples, matrix-related interferences were typically observed at the retention times of BBP, DEHP and DCHP, which means that these three phthalates could not univocally be identified and quantified in some of the investigated samples. To overcome these matrix interferences, alternative analytical methods and/or extraction techniques should be investigated for their applicability. For instance, a solid phase microextraction (SPME) method followed by gas chromatography and flame ionisation detection (GC-FID), as developed by Ye et al. (2009), to determine trace levels of phthalates (DEHP included) in beer could be used in further measurement campaigns. Furthermore, the analytical procedure proved to be inadequate for the determination of DiDP and DiNP in food products and packaging materials, because they were lost during extract purification and/or because retention times of DiNP and DiDP overlapped. Since these two substances are substitutes for DEHP (ECPI, 2010) and since a TDI of 150 µg kg^{-1} bw day^{-1} for DiNP and DiDP has been established by EFSA (2005d; 2005e), it would be valuable to improve the analytical procedure to adequately detect these two compounds in food products and packaging materials present on the Belgian market.

Currently, only little scientific information is available on the occurrence of phthalates – especially other ones than DEHP – in raw cow's milk and feed. In this PhD thesis, several hypotheses were put forward (see discussion section of Chapter II.4) to explain the observed differences in concentrations of phthalates in raw cow's milk between farms and between seasons. Mainly due to time constraints, it was not possible to verify these hypotheses. For instance, in this PhD study, manually obtained samples of raw cow's milk were gathered at two farms, but only during winter. The collection of corresponding samples during summer, would have allowed to investigate the role of feed during winter and summer on phthalate levels in raw cow's milk in more detail. Lastly, with respect to farm-specific influences, possible elements for future research might include the effect of differences between farms regarding the use and/or type of cleaning products, disinfectants, milk tubes/pipes and equipment in the stables (e.g. cribs) on phthalate levels in raw cow's milk.

Unlike for most chemical substances, the metabolites of phthalates have proven to be more toxic/bioactive than their parent compounds (Frederiksen et al., 2007; Koch and Calafat, 2009; Wittassek et al., 2011). For this reason, it might be interesting to (1) examine the metabolism of phthalates in cows, pigs, poultry and other animals in order to get a clear understanding of the transfer of phthalates and especially their metabolites into meat, milk and eggs and (2) to measure phthalate metabolites in animal products.

By investigating a contemporary Belgian milk production chain in depth, several contamination sources were identified at the farm (e.g. milking equipment and feed), industry (e.g. pasteurisation enhances migration) and retail level (e.g. the association between phthalate concentration and fat content). For further research, it would be interesting to follow a similar approach to identify

possible contamination sources for phthalates in bread, since both the monitoring and modelling intake assessment approach (Chapters II.3 and III.2, respectively) revealed that bread was a main contributor to the dietary exposure of phthalates in the Belgian adult population.

This PhD thesis was the first to investigate the effect of home-cooking on the presence of phthalates in foods. As food products can be prepared and consumed in many different ways (e.g. fried, baked, grilled, deep-fried, steamed and boiled), it is recommended that future research will expand to the investigation of other meat, fish and/or vegetable products and the effect of other cooking methods than the ones discussed in this study (Chapter II.6). Additionally, degradation products of phthalates (e.g. phthalic acid) formed during home-cooking processes should preferably be analysed as well.

Due to the absence of Belgian monitoring data of phthalates in media such as air, manure and sludge, this PhD study had to rely on European data of phthalates in air (gas phase, particle phase, total air and deposition), manure and sludge for the environmental fate modelling of phthalates with EN-forc (Chapter III.1). The use of non-Belgian data may have led to over- or underestimations of phthalate concentrations by the EN-forc model and thus strengthens the need for Belgian monitoring data of phthalates in media such as air, manure and sludge.

IV.4.2 Recommendations for model development

DEP, DnBP, BBP and DEHP levels in foliar crops, eggs, cow's milk and meat (e.g. beef and pork) as predicted by the EN-forc model corresponded well with measured phthalate concentrations (see Chapter III.1). Nevertheless, the current plant transfer models of Trapp and Legind (2011) that were implemented in the EN-forc model proved to be insufficient to accurately predict levels of DEP, DnBP and DEHP in pasture, root crops and/or tubers. Furthermore, model validation revealed that, to date, simple models to predict concentrations of organic chemicals in offal are lacking. So, for all these types of food products, further research is highly encouraged.

This PhD project clearly showed that the EN-forc model can be a suitable tool for describing the environmental transfer and for predicting the dietary exposure of DEP, DnBP, BBP and DEHP in Belgian foods and Belgian adults, respectively. Consequently, the EN-forc model can provide an inexpensive and rapid solution for the assessment of chemicals that are hard to analyse or for which chemical analyses are expensive or time-consuming. However, due to the absence of sufficient and reliable input data (both concentration and physico-chemical data), the EN-forc model was not able to predict the environmental transfer and dietary intake of phthalates like DiBP, DiNP and DiDP. Since these three compounds are all listed on the European priority list of chemicals with potential endocrine disrupting activities as Category 2 substances (European Commission, 2014b), future research on these compounds would be useful to accurately predict their environmental fate and resulting dietary exposure.

IV.4.3 Recommendations to assess phthalate exposure in the Belgian population

Since the original research questions of this PhD project focused on dietary exposure, non-dietary phthalate exposure routes were not investigated for the Belgian adult population, making it impossible to assess the overall exposure to phthalates. Nevertheless, for some phthalates, non-dietary exposure routes may contribute for a major part to the total exposure to phthalates. For

instance, Wormuth et al. (2006) revealed that up to 80% of the total daily exposure to DEP is caused by the use of personal care products (e.g. deodorant, perfume, aftershave and shampoo).

In Belgium, integral phthalate exposure has already been estimated for Flemish adolescents and for Belgian mother-child pairs by measuring phthalate metabolites in urine (Geens et al., 2014; The Belgian Steering Committee on HBM, 2013). By using the model of David (2000; see also Equation 1 in Section I.6.1.1), these phthalate metabolite levels could subsequently be converted to daily internal phthalate diester intakes. To date, there are no measurements available of urinary phthalate metabolite levels for other subgroups of the Belgian population (e.g. infants, adult men and elderly). Although giving a clear view of the integral phthalate exposure, this approach also has its limitations. By analysing phthalate metabolites in urine, total exposure to phthalates in the Belgian population is identified, but the contribution of each of the possible contamination routes (i.e. oral ingestion, inhalation, dermal absorption and parenteral exposure for medical devices) to the total exposure of phthalates is still unknown. To fill this knowledge gap, phthalates should be analysed in Belgian dust, soil, water and air samples and in food and consumer products (e.g. personal care products and toys) present on the Belgian market. Combining these measurements with Belgian survey/questionnaire data on personal lifestyle, food consumption and product use would allow to predict integral phthalate exposure in the Belgian population and gain insight in the importance of the various exposure routes.

Alternatively, integral exposure to phthalates could also be estimated for the Belgian population by using direct and/or indirect exposure models (see also Chapter I.6). To date, it would already be possible to predict the integral exposure of the Belgian population to DEHP in a direct way by making use of the PBPK (Physiologically based pharmacokinetic) model of Lorber et al. (2010). However, for other phthalates, PBPK models in humans are still missing. With respect to indirect exposure modelling, there currently does not exist a model that is capable to predict human phthalate exposure for all relevant exposure routes. The only option at the moment, is to use a combination of two or more indirect exposure models. This approach was already used by Müller et al. (2003), who used the EUSES model (Vermeire et al., 2005; 1997) in combination with the CONSEXPO model (van Veen, 2001) to predict integral exposure to DEHP, BBP, DiDP and DiNP in the Danish population.

IV.5 General conclusion

This PhD aimed to assess the dietary exposure of the Belgian adult population to eight phthalates: DMP, DEP, DiBP, DnBP, BBP, DCHP, DEHP and DnOP. For this purpose, two approaches were applied, differing in the nature of the phthalate concentration data in food products (i.e. measured *vs.* predicted). Both dietary intake assessment approaches revealed that Belgian adults are exposed daily to several phthalates, especially to DEHP, DiBP and DnBP. This observation confirms the findings of researchers who investigated the contribution of different routes (including the diet) to integral phthalate exposure, namely: food ingestion plays a crucial role in the integral human exposure to DiBP, DEHP and to some extent also to DnBP and BBP, but for phthalates such as DMP and DEP, this type of exposure route is rather negligible. The food groups contributing the most to the dietary exposure to phthalates in Belgium were grain and grain-based products (especially bread), meat and meat products, fruits and milk and dairy products. These food products are all frequently consumed – even daily – in relative high amounts by the majority of the Belgian population.

Evaluation of the estimated dietary exposure distribution rates against TDI values was only possible for the phthalates DEP, DnBP, BBP and DEHP. Taking into consideration the relative contributions of the diet to integral phthalate exposure, none of the corresponding TDI values were expected to be exceeded for the Belgian adult population. However, evaluating dietary exposures available for other Belgian subgroups revealted that, in younger age classes (i.e. for Belgian infants and preschool children), it is possible that in specific situations the TDI of DEHP will be exceeded. Many of the adverse health effects associated with DEHP (and other phthalates) exposure are related to endocrine disrupting and consequently also reproductive and developmental effects. This means that exposure to phthalates is the most critical during life periods such as pregnancy, infancy (including breast-feeding period), adolescence and senescense.

Besides estimating and evaluating dietary phthalate exposure, this PhD dissertation also investigated potential contamination sources (i.e. environmental transfer, the production process, packaging materials and processing methods) in order to be able to better understand the occurrence of phthalates in food products present on the Belgian market. Based on both the performed measurements and modelling studies, various trends could be observed and several hypotheses could be put forward. For instance, this project revealed that pasta and rice contained more phthalates when packed in printed and/or recycled cardboard than when packed in plastic and the milk campaign demonstrated that phthalates may migrate from the milking equipment during the mechanical milking process. At farm level, milk may also be contaminated with phthalates as a result of the ingestion of contaminated pasture by the cows. This feed product was – according to the performed model predictions – most likely contaminated with phthalates due to air gas phase exchange during cultivation.

In general, it can be concluded that this PhD thesis contributed in many ways to the scientific knowledge regarding population exposure to phthalates. For this reason, this thesis may serve both as a source of inspiration for policy makers in the context of guideline development, as well as a guide for future research focusing on particular contamination pathways of phthalates in foods and the role of other exposure routes of phthalates in humans.

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Summary

Introduction to phthalates

During the last two decades, several large-scale food safety incidents have been reported in the Belgian media, leading to widespread debates amongst policy makers and the general public. Many of these incidents were directly related to the unintentional presence of chemical substances to food products, such as the occurrence of polychlorinated biphenyls (PCBs) in animal feed products during the notorious dioxin crisis of 1999. These food incidents have led to a higher level of (chemical) food safety concern among Belgian citizens and have resulted in the safety of the Belgian food chain being a top-level priority for the Belgian government.

This PhD dissertation focuses on the presence of phthalates in food products as well as the resulting human exposure to these compounds. *Phthalates* is the common name for dialkyl or alkyl aryl esters of *ortho*-phthalic acid (1,2-benzene dicarboxylic acid). These organic, lipophilic chemical substances are mainly added to plastic polymers in order to increase flexibility, but – depending on the length of the alkyl chain – they may also be applied in printing inks, adhesives, solvents, personal care products, building materials, and so on. More than 30 different phthalate compounds are commercially available on the current European market and several of these compounds have been reported in literature to be present in food products as contaminant. Eight of them were considered in this thesis: dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), dicyclohexyl phthalate (DCHP), di(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DnOP).

Phthalates can enter the food chain via the environment as well as via migration from contact materials used during cultivation, production, storage or even during cooking at home. So, in order to identify all relevant contamination pathways for phthalates in food products and to be able to correctly assess dietary phthalate exposure of the Belgian adult population, several measurement and modelling studies were conducted during this doctoral project.

Analysing the occurrence of phthalates in food

To obtain high quality measurement data, an analytical procedure was needed for the determination of the eight considered phthalate compounds in food products and packaging materials present on the Belgian market. A method based on gas chromatography - low resolution - mass spectrometry combined with electron impact ionisation (GC-EI-MS) was developed and validated during the "PHTAL" project, a study funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (Contract No. RT/08/1 PHTAL) and performed by a consortium of the Flemish Institute for Technological Research (VITO) and Ghent University (Department of Public Health). During this project, a sample handling protocol was also set up to reduce the risk of contamination during sample preparation and analysis, since phthalates are omnipresent in the laboratory environment.

In the PHTAL project, phthalates were analysed in 591 food and 30 packaging samples. DEHP was the most detected phthalate compound, followed by DiBP, DnBP and BBP. On the contrary, DMP, DEP, DCHP and DnOP were rarely present in the investigated samples. In general, levels of DEHP were the highest of all phthalates in every investigated food group. The lowest phthalate concentrations were observed in milk and milk beverages, baby food, vegetarian food, eggs and aqueous-based

beverages. In packaging materials, especially in cardboard, the highest phthalate levels were observed for DiBP. By conducting concentration profiles (i.e. analysing phthalates in samples taken at different places of a food product), the PHTAL project also demonstrated the important role of food processing – over packaging – on the contamination of Belgian food products with phthalates. Furthermore, an in-depth analysis of different purchased bread samples revealed that phthalate contamination is highly location dependent. This led to the conclusion that phthalate containing contact materials (e.g. baking trays) and contaminated ingredients (e.g. flour) used during the production process of bread are probably the most important contributors to phthalate contamination in bread.

The influence of cooking at home (i.e. boiling, steaming, frying, deep-frying and grilling) on the phthalate levels in starchy products (potato, pasta and rice), meat, fish and vegetables was investigated in detail in a separate measurement campaign. After processing, the number of positive samples of all phthalates (except DMP and DiBP) generally declined, suggesting that home cooking processes reduce phthalate levels in food. With respect to meat and fish, levels were observed to be lower when fried in a frying pan with margarine than fried in a non-stick frying pan without margarine. This might indicate that phthalates migrated from the coating of the non-stick frying pan into the meat/fish and/or from the meat/fish into the margarine used during frying.

To investigate contamination pathways in more depth, one type of food chain – i.e. a Belgian contemporary milk production chain – was chosen to be further examined. To do this, milk and dairy product samples were collected at various stages of the production chain. At farm level, phthalate concentrations in raw cow's milk turned out to be varying among farms as well as over seasons. Overall, silage and pasture were observed to be the most important sources for DiBP and/or DEHP in raw cow's milk. During the mechanical milking process, additional contamination of milk with BBP and DEHP took place, probably as a result of the migration from contact materials such as plastic milking tubes. At industry level, heating steps like pasteurisation seemed to enhance DEHP, DiBP and DnBP migration to milk and milk powder. Packaging additionally contaminated milk powder at the factory with DEHP, DiBP, DnBP and BBP. Lastly, at retail level, a positive relationship between fat content and DEHP content in milk and dairy products was observed.

During the different measurement campaigns of this doctoral project, measured phthalate levels were generally in line with the levels observed in food products in other recent European studies. Compared to older European measurement data, differences in phthalate concentrations can be observed, especially regarding DEHP, for which a decreased trend was noticed. This observation could be explained by the fact that DEHP is more and more substituted by alternative plasticisers in the European Union.

Modelling the occurrence of phthalates in foods

Due to the omnipresence of phthalates, the analytical measurement of phthalates in food products is complex and time-consuming. To meet these difficulties, modelling could offer a rapid and rather inexpensive solution. In this PhD project, the multimedia model "EN-forc" (ENvironmental Food transfer model for ORganic Contaminants) was developed to predict the occurrence of DEP, DnBP, BBP and DEHP in a hundred Belgian agricultural products as a result of environmental transfer. In a second phase, additional models describing the impact of fat content, processing and packaging were

also implemented in the EN-forc model to obtain predicted DEP, DnBP, BBP and DEHP concentrations in 1,908 different packaged and/or processed Belgian food products.

Validation of the environmental transfer module of the EN-forc model revealed that for the majority of the considered media (i.e. soil, groundwater, drinking water, foliar crops, eggs, meat and milk), predicted phthalate concentrations were within one order of magnitude of the observed concentration levels. The occurrence of DEP, DnBP and DEHP in root crops and tubers was underestimated by the plant models implemented in EN-forc. Several reasons are possible for this. First, the input data (e.g. physic-chemical properties and background concentrations in environmental media) used in the different modules of EN-forc may not have been of sufficient quality. In various cases, EN-forc relied on non-Belgian input data due to a lack of Belgian figures (e.g. outdoor air concentrations were gathered from a French study). Second, it has to be considered that all models implemented in EN-forc have their own limitations, approximation errors and application ranges. Model validation also revealed that there currently are no appropriate models available in literature to predict the occurrence of phthalates in offal. This all opens ample opportunity for further research into the prediction of the environmental dynamics of phthalates.

Estimating dietary phthalate exposure in the Belgian adult population

Two intake assessment approaches were applied to estimate the dietary exposure to phthalates in the Belgian adult population. A first probabilistic approach was conducted in the PHTAL project and made use of measured concentration data of the eight considered phthalates in 572 food products. A second semi-probabilistic approach was conducted using the extended version of the EN-forc model and thus made use of predicted concentration data of DEP, DnBP, BBP and DEHP in 743 different food products. Both intake assessment approaches revealed that Belgian adults are exposed daily to several phthalates, especially to DEHP, DiBP and DnBP. The results indicate that Belgian men and young adults are generally more exposed to phthalates through the diet than women and older people, respectively. A possible explanation for these dissimilarities is the difference in dietary patterns between these gender and age categories. None of the estimated exposures exceeded the available tolerable daily intake (TDI) values and for the majority of the population, the intake of phthalates was even assessed to be far below the TDI. The food groups contributing the most to the dietary phthalate exposure in Belgium were grains and grain-based products (especially bread), meat and meat products, fruits and milk and dairy products.

The median and 95th percentile intake estimates of DEP, DnBP, BBP and DEHP in the Belgian adult population as predicted by the EN-forc model in a semi-probabilistic way using predicted phthalate concentrations, agreed well with the results from the upper bound probabilistic scenario conducted in the PHTAL project. This indicates that the EN-forc model can be useful as a semi-probabilistic modelling tool for the prediction and evaluation of the long-term human daily dietary intake of phthalates or, by extension, other organic chemicals that are hard to measure.

As also observed in the monitoring studies, calculated phthalate exposure rates were generally in line or lower compared to intake estimates reported in recent or older European studies, respectively. Again, the substitution of several phthalates by alternative chemicals in the production chain may be responsible for this.

Relevance to public health

Most of the phthalates considered in this PhD dissertation have been associated with endocrine disrupting activities. As a consequence, phthalate exposure is the most critical during pregnancy, infancy, adolescence and senescence, since in these periods of human life, the endocrine system is undergoing changes. Although this study focused on dietary phthalate exposure in Belgian adults, dietary exposure in other subgroups in the Belgian population for which data were available (i.e. infants and preschoolers) was also evaluated against exposure limit values. This was only possible for DEP, DnBP, BBP and DEHP. For three of these compounds (i.e. DnBP, BBP and DEHP), food ingestion is one of the most (for DEHP even the most) important pathways with regard to integral phthalate exposure. So, taking actions in order to decrease food contamination will certainly have an effect on the integral exposure to these phthalates. Considering the relative contribution of food ingestion to integral exposure, it could be stated that for Belgian adults, no TDI exceedances are to be expected with regard to DEP, DnBP, BBP and DEHP exposure. However, for Belgian infants and preschoolers, there might be a chance that – in specific situations – the TDI of DEHP will be exceeded.

Suggestions for policy development and future research

This PhD thesis contributes on several fronts to the overall scientific knowledge on phthalates. The results obtained in the monitoring and modelling studies may serve as a basis for policy makers in the context of guideline development. For instance, dietary intake assessments revealed that grains, meat, fruit, milk and derived products contribute the most to the dietary exposure to phthalates in the Belgian adult population, underlining the need to specify maximum residue levels of phthalates in those food products. Also, the fact that the presence of DiBP and DnBP in food products is associated with the use of cardboard packages and the migration from printing inks may be useful to policy makers, since there is currently no legislation at the European level limiting or restricting the migration of DiBP and DnBP in this type of applications.

Because the analysis of phthalates is complex, expensive and time consuming, not every possible contamination pathway could be investigated in detail during this PhD project. Nevertheless, the results obtained in both the measurement and modelling studies may serve as a guideline for future research, for example, indicating the types of food products, phthalate compounds or production processes, and so on, to investigate. As an example, this PhD thesis reveals the need for more research on the occurrence of diisononyl phthalate (DiNP) and diisodecyl phthalate (DiDP) – two DEHP substitutes – in foods and packaging materials present on the Belgian market, in order to obtain reliable estimates of the dietary exposure to these compounds in the Belgian population. Also, the production process of bread – especially at different types/locations of bakeries – would be useful to investigate in more depth in the future.

Samenvatting

Inleiding tot ftalaten

Gedurende de afgelopen twintig jaar werden verschillende grootschalige incidenten inzake voedselveiligheid gerapporteerd in de Belgische media, leidend tot uitgebreide discussies tussen beleidsmakers en de algemene bevolking. Vele van deze incidenten waren rechtstreeks gerelateerd aan de onbedoelde aanwezigheid van chemische stoffen in voedingsmiddelen, zoals de aanwezigheid van polychloorbifenylen (PCB's) in veevoeder gedurende de notoire dioxinecrisis van 1999. Deze voedselincidenten leidden tot een hogere graad van ongerustheid over (chemische) voedselveiligheid bij de Belgische bevolking en hadden tot gevolg dat de veiligheid van de Belgische voedselketen topprioriteit werd voor de Belgische regering.

Dit doctoraatsproefschrift focust op de aanwezigheid van ftalaten in voedingsmiddelen alsook op de resulterende humane blootstelling aan deze stoffen. *Ftalaten* is de algemene benaming voor de dialkyl- of alkylarylesters van *ortho*-ftaalzuur (1,2-benzeendicarboxylzuur). Deze organische, lipofiele chemische verbindingen worden hoofdzakelijk toegevoegd aan plastic polymeren ter verhoging van de flexibiliteit, maar – afhankelijk van de lengte van de alkylketen – kunnen ze ook toegepast worden in drukinkten, lijmen, solventen, verzorgingsproducten, bouwmaterialen, enzovoorts. Meer dan 30 verschillende ftalaten zijn commercieel beschikbaar op de huidige Europese markt en verschillende van deze componenten werden in de literatuur als contaminant in voeding gerapporteerd. Acht van deze stoffen werden beschouwd in deze thesis: dimethylftalaat (DMP), di-ethylftalaat (DEP), di-isobutylftalaat (DiBP), di-*n*-butylftalaat (DnBP), benzylbutylftalaat (BBP), dicyclohexylftalaat (DCHP), di(2-ethylhexyl)ftalaat (DEHP) en di-*n*-octylftalaat (DnOP).

Ftalaten kunnen de voedselketen binnendringen via het milieu alsook via de migratie van contactmaterialen gebruikt tijdens de teelt, productie, bewaring of zelf tijdens het koken thuis. Dus, om alle relevante contaminatieroutes voor ftalaten in voedingsmiddelen te identificeren en om ftalaatblootstelling via voeding door de Belgische volwassen bevolking correct te kunnen inschatten, werden verscheidene meet- en modelleerstudies uitgevoerd tijdens dit doctoraatsproject.

Analyseren van het voorkomen van ftalaten in voeding

Om meetdata van hoge kwaliteit te bekomen, was een analytische procedure nodig voor het bepalen van de acht beschouwde ftalaten in voedingsmiddelen en verpakkingsmaterialen aanwezig op de Belgische markt. Een methode gebaseerd op gaschromatografie – lage resolutie – massaspectrometrie in combinatie met elektronenimpactionisatie (GC-EI-MS) werd ontwikkeld en gevalideeerd tijdens het "PHTAL"-project, een studie gefinancierd door de Belgische Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu (Contractnr. RT/08/1 PHTAL) en uitgevoerd door een consortium van de Vlaamse Instelling voor Technologisch Onderzoek (VITO) en Universiteit Gent (vakgroep Maatschappelijke Gezondheidkunde). Tijdens dit project werd tevens een protocol opgesteld voor het behandelen van stalen om het risico van contaminatie te beperken tijdens de voorbereiding en analyse van de stalen, aangezien ftalaten alomtegenwoordig zijn in de labo-omgeving.

In het PHTAL-project werden ftalaten geanalyseerd in 591 voedings- en 30 verpakkingsstalen. DEHP was de meest gedetecteerde ftalaatcomponent, gevolgd door DiBP, DnBP en BBP. De ftalaten DMP, DEP, DCHP en DnOP waren daarentegen slechts zelden aanwezig in de onderzochte voedings-

groepen. De laagste ftalaatconcentraties werden teruggevonden in melk en melkdranken, babyvoeding, vegetarische voeding, eieren en watergebaseerde dranken. In verpakkingsmaterialen, voornamelijk karton, werden de hoogste ftalaatwaarden waargenomen voor DiBP. Via het opstellen van voedingsconcentratieprofielen (i.e. het analyseren van ftalaten in stalen genomen op verschillende plaatsen in een voedingsmiddel) toonde het PHTAL-project ook de belangrijke rol aan van voedselverwerking – boven verpakking – op de contaminatie van Belgische levensmiddelen met ftalaten. Verder wees een diepgaande analyse van verschillende aangekochte broodstalen uit dat ftalaatcontaminatie sterk afhankelijk is van de locatie. Dit leidde tot de conclusie dat ftalaatbevattende contactmaterialen (bv. bakvormen) en besmette ingrediënten (bv. bloem) gebruikt tijdens het productieproces van brood mogelijk de meest belangrijke bijdragers zijn tot ftalaatcontaminatie in brood.

De invloed van koken thuis (i.e. koken, stomen, bakken, frituren en braden) op ftalaatconcentraties in zetmeelrijke levensmiddelen (aardappel, pasta en rijst), vlees, vis en groenten werd in detail nagegaan in een aparte meetcampagne. Over het algemeen daalde het aantal positieve stalen van alle ftalaten (met uitzondering van DMP en DiBP) na het uitvoeren van een hittebehandeling, wat doet vermoeden dat koken thuis ftalaatconcentraties in voeding doet afnemen. Met betrekking tot vlees en vis werden lagere concentraties waargenomen wanneer deze voedingsmiddelen gebakken werden in een braadpan met margarine dan wanneer gebakken in een antikleefpan zonder margarine. Dit zou kunnen aantonen dat ftalaten migreren vanuit de antiaanbaklaag van de antikleefpan naar het/de onderzochte vlees/vis en/of van het/de vlees/vis naar de gebruikte margarine tijdens het bakken.

Om contaminatieroutes diepgaander na te gaan, werd één type voedselketen – i.e. een hedendaagse Belgische melkproductieketen – gekozen om verder onderzocht te worden. Om dit te kunnen doen, werden stalen van melk en zuivelproducten verzameld op verschillende plaatsen van de productieketen. Op boerderijniveau bleken ftalaatconcentraties in rauwe koemelk te variëren tussen boerderijen alsook over de seizoenen. Globaal gezien waren kuilvoer en gras de meest belangrijke bronnen voor DiBP en/of DEHP in rauwe koemelk. Tijdens het machinaal melkproces vond een bijkomende contaminatie van melk met BBP en DEHP plaats, vermoedelijk als gevolg van migratie vanuit contactmaterialen zoals plastic melkslangen. Op industrieel niveau leken verhittingsprocessen zoals pasteurisatie de migratie van DEHP, DiBP en DnBP naar melk en melkpoeder te versterken. Tijdens het verpakken werd melkpoeder bijkomend besmet met DEHP, DiBP, DnBP en BBP in de fabriek. Tot slot werd op winkelniveau een positief verband tussen vetgehalte en DEHP-gehalte in melk en zuivelproducten aangetoond.

Tijdens de verschillende meetcampagnes van dit doctoraatsproject waren gemeten ftalaat-concentraties meestal gelijkaardig aan gehaltes teruggevonden in voedingsmiddelen van andere recente Europese studies. In vergelijking met oudere Europese meetgegevens werden verschillen in ftalaatconcentraties geobserveerd, vooral met betrekking tot DEHP, waarvoor een afnemende trend werd opgemerkt. Deze waarneming zou verklaard kunnen worden door het feit dat DEHP in de Europese Unie meer en meer wordt vervangen door alternatieve weekmakers.

Modelleren van het voorkomen van ftalaten in voeding

Wegens de alomtegenwoordigheid van ftalaten is het analytisch meten van ftalaten in voedingsmiddelen complex en tijdrovend. Om deze problemen tegemoet te komen zou modelleren een snelle en enigszins goedkope oplossing kunnen bieden. In dit doctoraatsproject werd het multimediamodel "EN-forc" (ENvironmental Food transfer model for ORganic Contaminants; milieutransfermodel voor organische contaminanten in voeding) ontwikkeld om het voorkomen van DEP, DnBP, BBP en DEHP in een honderdtal Belgische boerderijproducten te voorspellen als gevolg van milieutransfer. In een tweede fase werden bijkomende modellen die de impact van vetgehalte, verwerking en verpakking beschouwen ook geïmplementeerd in het EN-forc-model om voorspelde concentraties van DEP, DnBP, BBP en DEHP in 1908 verschillende verpakte en/of verwerkte Belgische voedingsmiddelen te bekomen.

Validatie van de milieutransfermodule van het EN-forc-model toonde aan dat voor de meerderheid van de beschouwde media (i.e. bodem, grondwater, drinkwater, bladgewassen, eieren, vlees en melk), voorspelde ftlaatconcentraties binnen één grootteorde lagen van gemeten concentraties. Het voorkomen van DEP, DnBP en DEHP in wortel- en knolgewassen werd onderschat door de geïmplementeerde plantenmodellen van EN-forc. Verschillende redenen zijn hiervoor mogelijk. Eerst en vooral zou het kunnen dat de inputdata (bv. fysicochemische eigenschappen en achtergrondconcentraties in milieucompartimenten) gebruikt in de verschillende modules van EN-forc niet van voldoende kwaliteit waren. In verschillende gevallen maakte EN-forc gebruik van niet-Belgische inputdata door een gebrek aan Belgische data (bv. buitenluchtconcentraties werden overgenomen van een Franse studie). Ten tweede moet rekening genomen worden met het feit dat alle modellen geïmplementeerd in EN-forc hun eigen beperkingen, benaderingsfouten en toepassingsgebieden hebben. Verder toonde de modelvalidatie ook aan dat er momenteel geen geschikte modellen beschikbaar zijn in de literatuur voor het voorspellen van het voorkomen van ftalaten in orgaanvlees. Dit alles opent ruimschoots de gelegenheid om het voorspellen van de dynamiek van ftalaten in het milieu verder te onderzoeken.

Inschatten van de ftalaatblootstelling via voeding door de Belgische volwassen bevolking

Twee benaderingen werden toegepast om ftalaatblootstelling via voeding door de Belgische volwassen bevolking in te schatten. Een eerste probabilistische benadering werd uitgevoerd in het PHTAL-project en maakte gebruik van gemeten concentratiedata van de acht beschouwde ftalaten in 572 voedingsmiddelen. Een tweede semi-probabilistische benadering werd uitgevoerd met behulp van de uitgebreide versie van het EN-forc-model en maakte gebruik van voorspelde concentratiedata van DEP, DnBP, BBP en DEHP in 743 verschillende voedingsmiddelen. Beide benaderingen toonden aan dat Belgische volwassenen dagelijks blootgesteld worden aan verschillende ftalaten, vooral aan DEHP, DiBP en DnBP. De resultaten duidden aan dat Belgische mannen en jongvolwassenen over het algemeen hoger blootgesteld worden aan ftalaten via voeding dan respectievelijk vrouwen en ouderen. Een mogelijke verklaring voor deze verschillen is het verschil in de voedingspatronen van deze geslachts- en leeftijdscategorieën. Geen van de geschatte blootstellingen overschreed de beschikbare toelaatbare dagelijkse innamewaarden (TDI's) en voor de meerderheid van de bevolking lag de ftalaatinname zelfs ver onder de TDI. De voedingsgroepen die het meeste bijdragen aan

ftalaatblootstelling via voeding in België waren granen en graanproducten (voornamelijk brood), vlees en vleesproducten, fruit en melk en zuivelproducten.

De medianen en 95ste percentielen van de innameschattingen van DEP, DnBP, BBP en DEHP in de Belgische bevolking voorspeld op een semi-probabilistische manier met behulp van het EN-forc-model gebruik makend van voorspelde ftalaatconcentraties kwamen goed overeen met de resultaten van het probabilistische bovengrensscenario van het PHTAL-project. Dit wijst aan dat het EN-forc-model gebruikt kan worden als een semi-probabilistisch modelleerinstrument voor het voorspellen en evalueren van de humane dagelijkse ftalaatblootstelling via voeding op lange termijn of, bij uitbreiding, zelfs voor de blootstelling aan andere organische stoffen die moeilijk te meten zijn in voeding.

Net zoals aangetoond in de meetcampagnes, waren de berekende ftalaatblootstellingswaarden over het algemeen vergelijkbaar of lager in vergelijking met innamewaarden gerapporteerd in respectievelijk recente of oudere Europese studies. Opnieuw zou het kunnen dat de vervanging van verscheidene ftalaten door alternatieve chemische stoffen in de productieketen hiervoor verantwoordelijk zijn.

Relevantie voor volksgezondheid

De meeste ftalaten beschouwd in deze doctoraatsthesis werden gerelateerd aan hormoon-verstorende activiteiten. Bijgevolg is ftalaatblootstelling het meest cruciaal tijdens de zwangerschap, de eerste levensjaren, adolescentie en senescentie aangezien tijdens deze levensfasen het endocriene stelsel wijzigingen ondergaat. Ook al lag de nadruk van deze studie op ftalaat-blootstelling via voeding in Belgische volwassenen, toch werd blootstelling via voeding ook in andere groepen van de Belgische bevolking waarvoor data beschikbaar waren (zijnde zuigelingen en kleuters), geëvalueerd ten opzichte van drempelwaarden. Dit was enkel mogelijk voor DEP, DnBP, BBP en DEHP. Voor drie van deze stoffen (DEP, BBP en DEHP) is voeding één van de belangrijkste (voor DEHP zelfs de belangrijkste) routes met betrekking tot totale ftalaatblootstelling. Het ondernemen van acties om de contaminatie van voedingsmiddelen te doen afnemen zal dan ook vast en zeker een effect hebben op de totale blootstelling aan deze ftalaten. Rekening houdend met de relatieve bijdrage van voedselingestie tot de totale blootstelling aan ftalaten, kon vastgesteld worden dat er geen TDI-overschrijdingen te verwachten zijn voor de Belgische volwassen bevolking met betrekking tot DEP-, DnBP-, BBP- en DEHP-blootstelling. Echter, voor Belgische zuigelingen en kleuters bestaat de kans dat – in specifieke situaties – de TDI van DEHP zal overschreden worden.

Suggesties voor beleidsvorming and toekomstig onderzoek

Dit doctoraatsproefschrift draagt op verschillende manieren bij tot de globale wetenschappelijke kennis inzake ftalaten. De resultaten behaald in de meet- en modelleerstudies kunnen als basis dienen voor beleidsmakers in de context van beleidsvorming. Bijvoorbeeld, de innameschattingen toonden aan dat granen, vlees, fruit, melk en afgeleide producten het meeste bijdragen aan de ftalaatblootstelling via voeding door de Belgische volwassen bevolking, wat de behoefte benadrukt om maximale residuwaarden voor ftalaten in deze types voedingsmiddelen op te stellen. Ook het feit dat de aanwezigheid van DiBP en DnBP in voedingsmiddelen gerelateerd is aan het gebruik van kartonnen verpakkingen en aan de migratie vanuit drukinkten kan zinvolle informatie zijn voor

beleidsmakers aangezien er momenteel geen wetgeving bestaat op Europees niveau die de migratie van DiBP en DnBP in dit type toepassingen beperkt of verbiedt.

Aangezien de analyse van ftalaten complex, duur en tijdrovend is, kon niet elke mogelijke contaminatieroute in detail onderzocht worden tijdens dit doctoraatsproject. Desalniettemin kunnen de resultaten die bekomen werden in de meet- en modelleerstudies dienen als richtlijn voor verder onderzoek. Zo kunnen de resultaten bijvoorbeeld aangeven welke type voedingsmiddelen, ftalaatcomponenten, productieprocessen, en zo verder onderzocht kunnen worden. Ter illustratie, dit doctoraatsproefschift onderstreept de noodzaak naar meer onderzoek inzake de aanwezigheid van di-isononylftalaat (DiNP) en di-isodecylftalaat (DiDP) – twee vervangers voor DEHP – in voedingsmiddelen en verpakkingsmaterialen aanwezig op de Belgische markt, met het oog op het bekomen van betrouwbare schattingen van de blootstelling aan deze componenten via voeding door de Belgische bevolking. Ook het productieproces van brood – vooral voor verschillende types van bakkerijen en locaties – zou zinvol zijn om in de toekomst meer in detail onderzocht te worden.

Annexes

Table 53: Phthalate concentrations (min-max (median)) determined in every subgroup. Concentrations in foods and beverages are reported in µg/kg fresh weight *and concentrations in packaging materials in* ng/cm². *The number of samples is given between brackets.*

Group – Subgroup	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (27)								
Fruits (7)	ND-0.2 (ND)	ND-2.0 (1.7)	ND-8.8 (ND)	ND-3.1 (ND)	ND-26 (0.5)	ND-26 (12)	ND-0.5 (ND)	ND-0.4 (ND)
Vegetables (17)	ND-4.6 (ND)	ND	ND-4.8 (1.0)	ND-5.6 (1.7)	ND-9.0 (ND)	ND-1413 (ND)	ND-0.2 (ND)	ND-0.9 (ND)
Nuts (3)	ND	ND	4.1-13 (8.1)	5.6-17 (14)	9.8-20 (11)	31.0-74 (35)	ND	ND
Milk and dairy products (56)								
Milk (8)	ND	ND	ND-0.7 (ND)	ND-0.8 (ND)	ND	7.8-20 (14)	ND-4.6 (ND)	ND
Milk beverages (8)	ND-0.1 (ND)	ND-0.6 (ND)	0.3-0.9 (0.5)	ND-0.9 (0.6)	ND-1.7 (ND)	2.8-17 (6.3)	ND-2.9 (ND)	ND
Cheese (21)	ND	ND-5.3 (ND)	ND-116 (6.2)	ND-54 (4.6)	ND-8.2 (ND)	31-743 (148)	ND-42 (ND)	ND-3.7 (ND)
Fresh cheese, yoghurt, cream, dessert, etc. (19)	ND-0.5 (ND)	ND-11 (ND)	ND-29 (2.1)	ND-6.5 (2.0)	ND-4.2 (ND)	ND-260 (25)	ND-7.3 (0.4)	ND-5.7 (ND)
Cereals and cereal products (47)								
Bread (18)	ND-0.9 (ND)	ND-12 (ND)	ND-152 (7.6)	ND-13 (ND)	ND-8.1 (2.4)	ND-1073 (157)	ND-3.6 (ND)	ND-2.8 (0.3)
Breakfast cereals (7)	ND	ND-1.8 (0.9)	1.5-66 (17)	1.8-11 (4.9)	0.6-6.6 (1.8)	7.2-63 (37)	ND	ND
Pasta (11)	ND-1.4 (ND)	ND-558 (ND)	ND-212 (25)	ND-22 (4.3)	ND-5.8 (1.0)	6.9-673 (118)	ND-0.1 (ND)	ND-1.2 (0.1)
Rice (4)	ND-0.4 (ND)	1.5-88 (3.8)	ND-1054 (414)	4.6-43 (35)	ND-11 (2.3)	26-204 (46)	ND	ND
Flour, starches and oatmeal (7)	ND	0.5-3.6 (0.6)	2.0-252 (8.7)	1.9-61 (6.2)	ND-14 (ND)	5.1-48 (18)	ND	ND-0.5 (ND)
Meat and meat products (22)								
Meat (13)	ND-25 (5.0)	ND-1.4 (ND)	ND-9.7 (2.0)	ND-15 (2.4)	ND-18 (ND)	19-433 (41)	ND	ND-6.9 (ND)
Meat products (9)	ND-18 (1.2)	ND-1.0 (ND)	0.6-6.6 (2.0)	ND-1.7 (ND)	ND-12 (ND)	10-278 (46)	ND-2.0 (ND)	ND-51 (ND)
Fish and fish products (18)								
Fish (10)	ND-43 (ND)	ND-2.7 (0.7)	ND-12 (ND)	ND-8.6 (ND)	ND-8.0 (ND)	ND-5932 (29)	ND	ND
Fish products (6)	ND-1.5 (ND)	ND-9.3 (ND)	ND-13 (ND)	ND-2.9 (ND)	ND-3.1 (ND)	11.0-2596 (86)	ND	ND
Crustaceans (2)	ND-0.2	ND	ND-8.6	4.2-13	ND-1.6	145-2636	ND-0.1	ND-0.7
Fat and oils (26)								
Vegetable oils (15)	ND-32 (ND)	ND-154 (ND)	ND-53 (ND)	ND-203 (ND)	7.8-1127 (29)	ND-1827 (136)	ND-13 (ND)	ND
Vegetable fat (8)	ND	ND	ND-14 (ND)	ND	ND-20 (8.6)	ND-177 (ND)	ND	ND
Animal fat (3)	ND	ND	ND-12 (ND)	ND	ND-11 (ND)	125-508 (390)	ND	ND

Group – Subgroup	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Snacks (28)								
Salty biscuits (4)	ND	ND	4.7-56 (9.2)	ND-53 (5.8)	ND-10 (ND)	ND-308 (43)	ND	ND
Sweet biscuits and cakes (10)	ND-6.1 (ND)	ND-4.7 (ND)	1.9-114 (4.3)	2.6-33 (6.8)	ND-14 (2.7)	15-151 (50)	ND-4.7 (ND)	ND
Confectionery (4)	ND-2.8 (ND)	ND-5.3 (ND)	0.6-32 (3.8)	1.3-65 (4.2)	0.2-4.1 (1.2)	32-165 (82)	ND-1.5 (ND)	ND-73 (0.3)
Syrup, sugar, honey, popcorn, chocolate spread, etc. (10)	ND-0.3 (ND)	ND-2.4 (ND)	ND-17 (1.6)	ND-41 (1.9)	ND-1.7 (0.2)	ND-243 (5.6)	ND	ND-1.0 (ND)
Condiments and sauces (40)								
Condiments (7)	ND-4238 (11)	ND-84 (16)	ND-155 (32)	ND-157 (6.6)	ND-388 (1.5)	0.1-2154 (54)	ND-0.7 (ND)	ND-120 (0.2)
Pesto (4)	ND	ND	15-22 (21)	10-52 (18)	4.4-85 (24)	80-532 (148)	ND	ND-3.0 (ND)
Mayonnaise (6)	ND	ND	ND	ND-17 (11)	ND-23 (ND)	21-320 (64)	ND	ND
Mustard, vinaigrette, ketchup, curry, etc. (23)	ND-21 (ND)	ND-11 (ND)	ND-18 (1.3)	ND-105 (1.3)	ND-21 (2.2)	ND-100 (12)	ND-2.8 (ND)	ND-4.0 (ND)
Miscellaneous (22)								
Ready-to-eat meals (22)	ND-4.7 (ND)	ND-2.5 (ND)	ND-344 (3.3)	ND-28.0 (3.4)	ND-5.9 (0.8)	ND-718 (16)	ND-1.8 (ND)	ND-2.6 (ND)
Baby food (17)								
Milk powder (3)	ND-0.2 (ND)	ND-0.2 (ND)	2.7-4.9 (3.6)	1.7-6.6 (2.5)	1.9-16 (16)	37-62 (42)	ND-1.8 (0.8)	0.3-3.0 (1.1)
Fruit puree, vegetable puree, soup, etc. (14)	ND-0.1 (ND)	ND-1.6 (0.1)	0.1-16 (2.4)	0.1-32 (0.8)	ND-15 (2.0)	ND-67 (21)	ND-0.3 (ND)	ND-1.2 (0.1)
Beverages (85)								
Beer (18)	ND-0.1 (0.1)	ND-0.1 (0.1)	ND-1.2 (0.1)	ND-0.6 (0.2)	ND-1.6 (ND)	ND	ND-0.1 (ND)	ND-0.1 (ND)
Soft drinks (25)	ND-0.1 (0.1)	ND-0.2 (ND)	ND-1.8 (0.1)	ND-0.9 (0.1)	ND-0.2 (0.1)	0.1-11.0 (1.2)	ND-0.1 (ND)	ND-0.8 (ND)
Juices (22)	ND-0.2 (0.1)	ND-0.3 (0.1)	0.1-1.4 (0.3)	ND-2.1 (0.3)	ND-1.5 (0.1)	ND-4.9 (0.7)	ND-0.1 (ND)	ND-0.1 (ND)
Water (20)	ND	ND	ND-2.0 (ND)	ND-0.2 (ND)	ND-0.1 (ND)	ND-0.1 (ND)	ND	ND
Packaging materials (12)								
Cardboard (5)	ND-0.4 (ND)	ND-41 (11)	24-523 (162)	12-96 (38)	0.7-24 (4.3)	38-319 (121)	ND-25 (1.0)	ND-1.5 (ND)
Tetra brick (2)	ND-0.1	ND	14-33	23-89	0.8-1.4	11-26	ND-0.7	ND-0.7
Plastic (5)	ND-0.3 (ND)	ND-8.0 (0.7)	ND-14 (7.5)	ND-20 (2.6)	ND-4.6 (0.5)	1.1-53 (18)	ND-1.2 (ND)	ND

ND: not detected.

Table 54: Phthalate concentrations (min-max (median)) determined in every subgroup for the two measurement campaigns together. Concentrations in foods and beverages are reported in µg/kg fresh weight and concentrations in packaging materials in ng/cm². The number of samples is given between parentheses.

Group – Subgroup	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Fruits and vegetables (47)								
Fruits (17)	ND-0.8 (ND)	ND-26 (1.8)	ND-480 (ND)	ND-18 (ND)	ND-58 (0.5)	ND-361 (16)	ND-0.5 (ND)	ND-9.5 (ND)
Vegetables (24)	ND-5.3 (ND)	ND-2.8 (ND)	ND-4.8 (1.0)	ND-5.6 (1.5)	ND-9.0 (ND)	ND-1413 (ND)	ND-0.4 (ND)	ND-3.0 (ND)
Nuts (6)	ND-13 (ND)	ND-889 (ND)	4.1-139 (11)	5.6-38 (13)	1.0-36 (10)	31-696 (61)	ND-1.4 (ND)	ND
Milk and dairy products (79)								
Milk (9)	ND	ND-1.3 (ND)	ND-0.7 (ND)	ND-0.8 (ND)	ND	7.8-20 (13)	ND-4.6 (1.4)	ND
Milk beverages (10)	ND-0.1 (ND)	ND-1.0 (ND)	0.3-1.6 (0.6)	ND-2.5 (0.6)	ND-1.7 (ND)	ND-19 (6.3)	ND-2.9 (ND)	ND
Cheese (38)	ND-1.7 (ND)	ND-5.3 (ND)	ND-116 (7.6)	ND-54 (4.6)	ND-48 (ND)	31-2385 (245)	ND-42 (ND)	ND-3.7 (ND)
Fresh cheese, yoghurt, cream, dessert, etc. (22)	ND-0.5 (ND)	ND-11 (ND)	ND-29 (2.0)	ND-6.5 (1.9)	ND-4.2 (ND)	ND-260 (24.5)	ND-7.3 (ND)	ND-5.7 (ND)
Cereals and cereal products (129)								
Bread (62)	ND-1.8 (ND)	ND-23 (1.6)	ND-871 (4.2)	ND-106 (3.8)	ND-8.1 (0.8)	ND-2264 (71)	ND-3.6 (ND)	ND-5.4 (ND)
Breakfast cereals (7)	ND	ND-1.8 (0.9)	1.5-66 (17)	1.8-11 (4.9)	0.6-6.6 (1.8)	7.2-63 (37)	ND	ND
Pasta (21)	ND-1.4 (ND)	ND-558 (0.3)	ND-212 (4.4)	ND-22 (2.2)	ND-5.8 (0.6)	ND-673 (40)	ND-0.1 (ND)	ND-1.2 (ND)
Rice (19)	ND-0.8 (ND)	ND-88 (1.5)	ND-1054 (40)	ND-90 (12)	ND-17 (3.7)	ND-1628 (130)	ND-0.5 (ND)	ND-0.9 (ND)
Flour, starches and oatmeal, popcorn (20)	ND-3.8 (ND)	ND-39 (0.9)	2.0-1383 (12)	ND-79 (7.4)	ND-24 (0.2)	ND-746 (18)	ND-1.6 (ND)	ND-6.1 (ND)
Meat and meat products (37)								
Meat (17)	ND-25 (ND)	ND-1.4 (ND)	ND-9.7 (1.2)	ND-15 (1.5)	ND-18 (ND)	ND-433 (29)	ND	ND-6.9 (ND)
Meat products (20)	ND-26 (1.1)	ND-11 (ND)	0.6-36 (4.7)	ND-25 (1.5)	ND-12 (ND)	10-850 (47)	ND-2.0 (ND)	ND-51 (ND)
Fish and fish products (22)								
Fish (14)	ND-43 (ND)	ND-2.7 (0.6)	ND-12 (ND)	ND-8.6 (ND)	ND-8.0 (ND)	ND-5932 (29)	ND	ND
Fish products (6)	ND-1.5 (ND)	ND-9.3 (ND)	ND-13 (ND)	ND-2.9 (ND)	ND-3.1 (ND)	11-2596 (15)	ND	ND-0.8 (ND)
Crustaceans (2)	ND-0.2 (ND)	ND	ND-8.6 (ND)	4.2-13 (8.6)	ND-1.6 (ND)	145-2636 (1391)	ND-0.1 (ND)	ND-0.7 (ND)
Fat and oils (34)								
Vegetable oils (21)	ND-32 (ND)	ND-154 (ND)	ND-53 (ND)	ND-203 (ND)	7.8-1127 (29)	ND-1827 (136)	ND-13 (ND)	ND
Vegetable fat (9)	ND	ND	ND-14 (ND)	ND	ND-20 (8.0)	ND-177 (ND)	ND	ND
Animal fat (4)	ND	ND-32 (ND)	ND-12 (ND)	ND	ND-13 (3.0)	125-508 (346)	ND	ND

Group – Subgroup	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Snacks (29)								
Salty biscuits (4)	ND	ND	4.7-56 (9.2)	ND-53 (5.8)	ND-10 (ND)	ND-308 (43)	ND	ND
Sweet biscuits and cakes (10)	ND-6.1 (ND)	ND-4.7 (ND)	1.9-114 (4.3)	2.6-33 (6.8)	ND-14 (2.7)	15-151 (50)	ND-4.7 (ND)	ND
Confectionery (5)	ND-2.8 (ND)	ND-5.3 (ND)	0.6-32 (4.7)	1.3-65 (5.1)	0.2-14 (1.6)	32-165 (85)	ND-1.5 (0.7)	ND-73 (0.3)
Syrup, sugar, honey, popcorn, chocolate spread, etc. (10)	ND-1.1 (ND)	ND-2.4 (ND)	ND-17 (1.6)	ND-41 (1.9)	ND-23 (0.2)	ND-243 (5.6)	ND-0.4 (ND)	ND-1.0 (ND)
Condiments and sauces (41)								
Condiments (7)	ND-4238 (11)	ND-84 (16)	ND-155 (32)	ND-157 (6.6)	ND-388 (1.5)	0.1-2154 (54)	ND-0.7 (ND)	ND-120 (0.2)
Pesto (4)	ND	ND	15-22 (21)	10-52 (18)	4.4-85 (24)	80-532 (148)	ND	ND-3.0 (ND)
Mayonnaise (7)	ND	ND	ND	ND-17 (10)	ND-23 (ND)	21-320 (57)	ND	ND-3.8 (ND)
Mustard, vinaigrette, ketchup, curry, etc. (23)	ND-21 (ND)	ND-11 (ND)	ND-18 (1.3)	ND-105 (1.3)	ND-21 (2.2)	ND-100 (12)	ND-2.8 (ND)	ND-4.0 (ND)
Miscellaneous (56)								
Ready-to-eat meals raw / cold (39)	ND-4.7 (0.1)	ND-109 (ND)	ND-344 (2.9)	ND-55 (4.0)	ND-5.9 (0.7)	ND-718 (22)	ND-1.8 (ND)	ND-7.0 (ND)
Ready-to-eat meals prepared (17)	ND-7.6 (0.3)	ND-143 (0.8)	0.7-20 (2.2)	ND-33 (2.7)	ND-5.6 (0.8)	ND-116 (19)	ND-0.6 (ND)	ND-15 (ND)
Baby food (17)								
Milk powder (3)	ND-0.2 (ND)	ND-0.2 (ND)	2.7-4.9 (3.6)	1.7-6.6 (2.5)	1.9-16 (16)	37-62 (42)	ND-1.8 (0.8)	0.3-3.0 (1.1)
Fruit puree, vegetable puree, soup, etc. (14)	ND-0.1 (ND)	ND-1.6 (0.1)	0.1-16 (2.4)	0.1-32 (0.8)	ND-15 (2.0)	ND-67 (21)	ND-0.3 (ND)	ND-1.2 (0.1)
Beverages (89)								
Beer (18)	ND-0.1 (0.1)	ND-0.1 (0.1)	ND-1.2 (0.1)	ND-0.6 (0.2)	ND-1.6 (ND)	ND	ND-0.1 (ND)	ND-0.1 (ND)
Soft drinks (25)	ND-0.1 (0.1)	ND-0.2 (ND)	ND-1.8 (0.1)	ND-0.9 (0.1)	ND-0.2 (0.1)	0.1-11 (1.2)	ND-0.1 (ND)	ND-0.8 (ND)
Juices (22)	ND-0.2 (0.1)	ND-0.3 (0.1)	0.1-1.4 (0.3)	ND-2.1 (0.3)	ND-1.5 (0.1)	ND-4.9 (0.7)	ND-0.1 (ND)	ND-0.1 (ND)
Water (20)	ND	ND	ND-2.0 (ND)	ND-0.2 (ND)	ND-0.1 (ND)	ND-0.1 (ND)	ND	ND
Coffee / tea (2)	0.1-0.1 (0.1)	ND-0.1 (ND)	0.1-0.2 (0.2)	ND	0.1-0.1 (0.1)	0.1-0.3 (0.2)	0.1-0.1 (0.1)	ND-0.1 (ND)
Wine (2)	0.1-0.1 (0.1)	ND-0.1 (ND)	ND-0.4 (ND)	4.7-30 (17)	0.4-21 (11)	ND-0.1 (ND)	ND-0.1 (ND)	ND-0.2 (ND)
Vegetarian food (5)								
Meat substitute (3)	ND-4.6 (0.6)	ND-2.4 (0.7)	4.1-7.5 (6.3)	ND-6.4 (6.2)	ND-12 (ND)	1.0-76 (13)	ND-2.7 (ND)	ND-3.3 (ND)
Milk product substitute (2)	ND	0.7-1.1 (0.9)	3.0-4.3 (3.7)	0.8-2.6 (1.7)	ND-0.9 (ND)	9.8-13 (11)	ND	ND
Eggs (2)	ND	ND	ND	ND	ND	ND	ND	ND
Boiling water (pasta / rice) (4)	ND-0.1 (0.1)	0.2-32 (1.8)	0.1-16 (2.6)	0.1-3.2 (0.9)	ND-1.1 (0.1)	ND-12.0 (1.1)	ND-0.1 (ND)	ND-0.1 (ND)

Group – Subgroup	DMP	DEP	DiBP	DnBP	BBP	DEHP	DCHP	DnOP
Packaging materials (30)								
Cardboard (7)	ND-0.4 (ND)	ND-49 (11)	24-523 (126)	12-96 (48)	0.7-28 (15)	38-482 (241)	ND-25 (2.4)	ND-2.0 (ND)
Tetra brick (2)	ND-0.1 (ND)	ND	14-33 (24)	23-89 (56)	0.8-1.4 (1.1)	11-26 (19)	ND-0.7 (ND)	ND-0.7 (ND)
Plastic (9)	ND-0.3 (ND)	ND-8.0 (ND)	ND-6.9 (18.0)	ND-22 (3.1)	ND-4.6 (0.6)	1.1-70 (24)	ND-2.9 (ND)	ND
Multi-layer (5)	ND	ND	1.0-5.0 (3.1)	ND-4.1 (3.2)	ND-0.6 (ND)	6.9-20 (11)	ND	ND
Paper (6)	ND	ND	1.4-12 (5.7)	3.2-66 (6.3)	ND-7.8 (0.6)	27-56 (36)	ND	ND
Wax (1)	ND	17	1.8	0.8	0.7	212	0.4	0.3

ND: not detected.

Base equations used in the different EN-forc modules

Chemical module

(1) $\log K_{oc} = a * \log K_{ow} + b$

(2) $K_d = (OC * K_{oc}) / 1000$

(3) $H' = H(T) / (R * T)$

(4) $H(T) = [P(T) * M] / S(T)$

(5) $D_a = 315.36 * \sqrt{(76 / M)}$

(6) $D_w = 0.0315 * \sqrt{(76 / M)}$

Soil module

(7) $dC / dt = -k * C + I$

(8) $k_v = [2 * D_{eff} * \theta_a * H'] / [d^2 * (\theta_w + \rho_s * K_d + \theta_a * H')]$

(9) $k_r = A_s / (\rho_s * d)$

(10) $k_{p,foliar} = TSCF * [Q / (Y * DM/100)] * [\rho_s / (K_d * [(\theta_w + \theta_a * H')/ K_d + \rho_s])]$

(11) $k_{p,root} = [Q / (Y * DM/100)] * [\rho_s / (K_d * [(\theta_w + \theta_a * H')/ K_d + \rho_s])]$

(12) $k_{p,tuber} = k_{dep} * [K_{PW} / (1000 * DM/100)] * [\rho_s / (K_d * [(\theta_w + \theta_a * H')/ K_d + \rho_s])]$

(13) $k_b = (k_{wb} * \theta_w) / (\theta_w + \rho_s * K_d + \theta_a * H')$

(14) $k_l = q / [d * (\theta_w + \rho_s * K_d + \theta_a * H')]$

(15) $I_A = [F_p * (1 – I_v)] / [\rho_s * d]$

(16) $I_M = [Q_M * C_M] / [\rho_s * d]$

(17) $I_S = [Q_S * C_S] / [\rho_s * d]$

(18) $C_{s,s} = (C * \rho_s) / [((\theta_w + \theta_a * H') / K_d) + \rho_s]$

(19) $C_{w,s} = C_{s,s} / K_d$

(20) $C_{a,s} = H' * C_{w,s}$

Water module

(21) $C_{gw} = [(C_{w,s} * L * q) + (k * i * M_z * C_{bg,gw})] / [(k_{hc} * i * M_z) + (L * q)]$

(22) $C_{wp} = C_{bg,wp} + [(2 * D_p * C_{w,s} * (dt/24) * \pi * r^2 * L_p) / (r * d_e * Q_{dw})]$

Air module

(23) $C_{p,a} = \phi * C_{a,a}$

(24) $C_{g,a} = (1 – \phi) * C_{a,a}$

(25) $F_p = C_{p,a} * (V_d + R_n * R_w * W_p)$

Plant module

(26) $dC_{p,foliar} / dt = -\alpha * C_{p,foliar} + k_{p,foliar} * C + [(G_{PL} * A * C_{g,a}) / (Y * (DM/100))]$

(27) $dC_{p,root} / dt = -[\alpha + (Q * 1000) / (Y * K_{RW})] * C_{p,root} + k_{p,root} * C$

(28) $dC_{p,tuber} / dt = -(\alpha + k_{dep}) * C_{p,tuber} + k_{p,tuber} * C$

(29) $C_{dep} = [F_p * I_v * (1 – \exp(-k_w * t_{growth}))] / [k_w * (Y * (DM/100))]$

(30) $C_{spl} = TF_{net} * [C_{s,s} + C_{w,s} * (\theta_w / \rho_s)]$

Animal module

(31) $J_{f,year} = J_{f,summer} * t_{f,summer} + J_{f,winter} * t_{f,winter}$

(32) $J_{f,summer/winter} = J_{pasture} + J_{feed} + J_{concentrate} + RBA_{soil} * J_{soil} + J_{water} + J_{milk}$

(33) $J_x = q_x * C_x$

(34) $C_y = BTF_y * J_{f,year}$

Abbreviations

α: plant loss rate due to metabolisation, photodegradation, volatilisation and/or growth dilution (yr^{-1})

φ: fraction adsorbed on atmospheric aerosol particles (-)

ρ_s: soil dry bulk density (kg dm m^{-3})

ϑ_a: volumetric soil air content (-)

ϑ_w: volumetric soil water content (-)

a: regression constant (0.49 for phthalates and 0.81 for dioxins)

A: plant surface area (m^2 m^{-2})

A_s: erosion loss (kg dm m^{-2} yr^{-1})

b: regression constant (1.05 for phthalates and 0.1 for dioxins)

BTF_y: biotransfer factor of contaminant to meat, liver, kidney, milk or eggs ((mg kg^{-1}) (mg $day^{-1})^{-1}$)

C: total soil concentration (mg kg^{-1} dm)

$C_{a,a}$: total contaminant concentration in air (mg m^{-3})

$C_{a,s}$: contaminant concentration in soil air phase (mg m^{-3})

$C_{bg,gw}$: background contaminant concentration in groundwater (mg m^{-3})

$C_{bg,wp}$: background contaminant concentration in drinking water (mg m^{-3})

C_{dep}: contaminant concentration due to particle deposition (mg kg^{-1} dm)

$C_{g,a}$: contaminant concentration in air gas phase (mg m^{-3})

C_{gw}: contaminant concentration in groundwater (mg m^{-3})

C_M: contaminant concentration in manure (mg kg^{-1} dm)

$C_{p,a}$: contaminant concentration in air particles (mg m^{-3})

$C_{p,foliar}$: contaminant concentration in foliar crops due to plant uptake and deposition of the gas phase (mg kg^{-1} dm)

$C_{p,root}$: contaminant concentration in root crops due to plant uptake (mg kg^{-1} dm)

$C_{p,tuber}$: contaminant concentration in tubers due to plant uptake (mg kg^{-1} dm)

C_S: contaminant concentration in sludge (mg kg^{-1} dm)

C_{spl}: contaminant concentration due to splashed soil particles (mg kg^{-1} dm)

$C_{s,s}$: contaminant concentration in soil solid phase (mg kg^{-1} dm)

C_{wp}: contaminant concentration in drinking water (mg m^{-3})

$C_{w,s}$: contaminant concentration in soil pore water phase (mg m^{-3})

C_x: contaminant concentration in pasture, feed, concentrate, soil, water or milk ((mg kg^{-1} dm) or (mg m^{-3}))

C_y: contaminant concentration in meat, liver, kidney, milk or eggs (mg kg^{-1})

d: soil thickness (m)

d_e: wall thickness of drinking water pipe (0.0027 m)

dt: standard stagnation time in pipe (24 h day^{-1})

D_a: diffusion coefficient in air (m^2 yr^{-1})

D_{eff}: effective diffusion coefficient in soil air phase ($m^2\ yr^{-1}$)
DM: dry matter content of plant (% of fresh weight)
D_p: permeation coefficient of plastic drinking water pipe ($m^2\ day^{-1}$)
D_w: diffusion coefficient in water ($m^2\ yr^{-1}$)
F_p: particle deposition flux ($mg\ m^{-2}\ yr^{-1}$)
G_{PL}: conductance of the leaf ($m\ yr^{-1}$)
H': dimensionless Henry coefficient (-)
$H(T)$: Henry coefficient at temperature T ($Pa\ m^3\ mol^{-1}$)
i: hydraulic gradient (0.001 $m\ m^{-1}$)
I: contaminant load to soil ($mg\ kg^{-1}\ dm\ yr^{-1}$)
I_A: contaminant load to soil due to atmospheric deposition ($mg\ kg^{-1}\ dm\ yr^{-1}$)
I_M: contaminant load to soil due to the use of manure ($mg\ kg^{-1}\ dm\ yr^{-1}$)
I_S: contaminant load to soil due to the use of sludge ($mg\ kg^{-1}\ dm\ yr^{-1}$)
I_v: fraction of particles intercepted by vegetation (-)
$J_{concentrate}$: daily contaminant intake via concentrate ($mg\ day^{-1}$)
J_{feed}: daily contaminant intake via feed ($mg\ day^{-1}$)
$J_{f,summer/winter}$: daily contaminant intake during summer/winter ($mg\ day^{-1}$)
$J_{f,year}$: contaminant intake on a yearly basis ($mg\ day^{-1}$)
J_{milk}: daily contaminant intake via milk ($mg\ day^{-1}$);
$J_{pasture}$: daily contaminant intake via pasture ($mg\ day^{-1}$)
J_{soil}: daily contaminant intake via soil ($mg\ day^{-1}$)
J_{water}: daily contaminant intake via water ($mg\ day^{-1}$)
J_x: daily contaminant intake via pasture, feed, concentrate, soil, water or milk ($mg\ day^{-1}$)
k: contaminant loss from soil (yr^{-1})
k_b: soil biodegradation constant (yr^{-1})
k_{dep}: depuration rate (yr^{-1})
k_{hc}: hydraulic conductivity of phreatic groundwater layer ($365\ m\ yr^{-1}$)
k_l: leaching coefficient (yr^{-1})
$k_{p,foliar}$: uptake coefficient for foliar crops (yr^{-1})
$k_{p,root}$: uptake coefficient for root crops (yr^{-1})
$k_{p,tuber}$: uptake coefficient for tubers (yr^{-1})
$k_{r:}$ run-off coefficient (yr^{-1})
k_v: volatilisation coefficient (yr^{-1})
k_w: plant weathering constant ($17.885\ yr^{-1}$)
k_{wb}: water biodegradation constant (yr^{-1})
K_d: soil-water partition coefficient ($m^3\ kg^{-1}$)
K_{oc}: organic carbon-water partition coefficient ($L\ kg^{-1}$)
K_{ow}: octanol-water partition coefficient (-)
K_{PW}: partition coefficient between potato and water ($L\ kg^{-1}$ fw)
K_{RW}: partition coefficient between root and soil pore water ($L\ kg^{-1}$)
L: length of groundwater plume (50 m)
L_p: total pipe length (50 m)
M: molecular mass ($g\ mol^{-1}$)
M_z: thickness of mixing zone (m)
OC: fraction of organic carbon in soil (-)

$P(T)$: vapour pressure at temperature T (Pa)
q: infiltration rate (0.265 m yr^{-1})
q_x: daily consumption of pasture, feed, concentrate, soil, water or milk ((kg dm day^{-1}) or (m^3 day^{-1}))
Q: transpiration rate (0.438 m^3 m^{-2} yr^{-1} for grains and fruits and 0.365 m^3 m^{-2} yr^{-1} for other foliar crops and root crops)
Q_{dw}: daily drinking water use (m^3 day^{-1})
Q_M: application rate of manure (kg dm m^{-2} yr^{-1})
Q_S: application rate of sludge (kg dm m^{-2} yr^{-1})
r: internal radius of drinking water pipe (0.0098 m)
R: universal gas constant (8.31451 Pa m^3 mol^{-1} K^{-1})
RBA_{soil}: relative bioavailability of contaminants in soil versus feed (-)
R_n: annual rainfall (m yr^{-1})
R_w: fraction retained after rainfall (1.00)
$S(T)$: water solubility at temperature T (mg L^{-1})
t: time (yr)
$t_{f,summer/winter}$: time fraction for summer/winter diet (-)
t_{growth}: growth period (yr)
T: temperature (K)
TF_{net}: net transfer of particles to the plant (-)
$TSCF$: transpiration stream concentration factor (-)
V_d: dry particle deposition rate (315725 m yr^{-1})
W_p: volumetric washout factor for particles (-)
Y: plant yield (kg fw m^{-2} yr^{-1})

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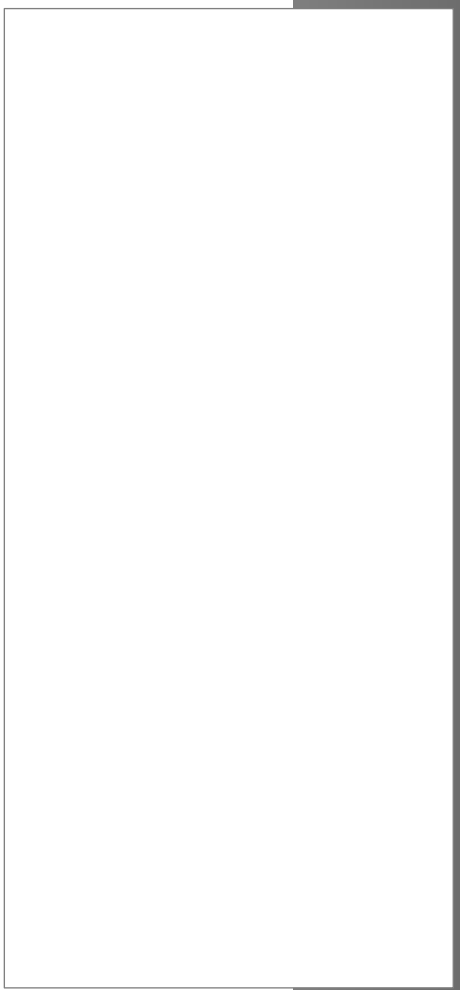
Tine

About the author



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Oct 2009	Course “Introduction to toxicology and ecotoxicology as the scientific basis for management of chemical risk”, Beltox, Elewijt, Belgium

Articles in international peer-reviewed journals included in Science Citation Index (A1)

Van Holderbeke, M., Geerts, L., Vanermen, G., Servaes, K., Sioen, I., De Henauw, S. & Fierens, T. (2014). Determination of contamination pathways of phthalates in food products sold on the Belgian market. *Environmental Research*, *134*, 345–352.

Fierens, T., Standaert, A., Cornelis, C., Sioen, I., De Henauw, S., Willems, H., Bellemans, M., De Maeyer, M. & Van Holderbeke, M. (2014). A semi-probabilistic modelling approach for the estimation of dietary exposure to phthalates in the Belgian adult population. *Environment International*, *73*, 117-127.

Geens, T., Bruckers, L., Covaci, A., Schoeters, G., Fierens, T., Sioen, I., Vanermen, G., Baeyens, W., Morrens, B., Loots, I., Nelen, V., Nemery de Bellevaux, B., Van Larebeke, N. & Den Hond, E. (2014). Determinants of bisphenol A and phthalate metabolites in urine of Flemish adolescents. *Environmental Research*, *134*, 110-117.

Fierens, T., Cornelis, C., Standaert, A., Sioen, I., De Henauw, S. & Van Holderbeke, M. (2014). Modelling the environmental transfer of phthalates and polychlorinated dibenzo-p-dioxins and dibenzofurans into agricultural products: the EN-forc model. *Environmental Research*, *133,* 282-293.

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S. & Sioen, I. (2013). Transfer of eight phthalates through the milk chain - A case study. *Environment International, 51*, 1-7.

Sioen, I., Fierens, T., Van Holderbeke, M., Geerts, L., Bellemans, M., De Maeyer, M., Servaes, K., Vanermen, G., Boon, P.E. & De Henauw, S. (2012). Phthalates dietary exposure and food sources for Belgian preschool children and adults. *Environment International, 48*, 102-108.

Fierens, T., Vanermen, G., Van Holderbeke, M., De Henauw, S. & Sioen, I., (2012). Effect of cooking at home on the levels of eight phthalates in foods. *Food and Chemical Toxicology, 50*, 4428-4435.

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S. & Sioen, I. (2012). Phthalates in Belgian cow's milk and the role of feed and other contamination pathways at farm level. *Food and Chemical Toxicology, 50*, 2945-2953.

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I. & Vanermen, G. (2012). Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology, 50*, 2575-2583.

Attended conferences and workshops

European human biomonitoring projects (DEMO)COPHES: results – workhop about phthalates
Nov 29, 2012, Brussels, Belgium (oral presentation + panel member)

5th ILSI International Symposium on Food Packaging
Nov 14-16, 2012, Berlin, Germany (oral presentation)

Innovation for sustainable production (i-SUP) 2012
May 6-9, 2012, Bruges, Belgium (oral + poster presentation)

BNS 3rd Annual meeting: Behaviour and nutrition, New insights for better solutions?
April 20, 2012, Brussels, Belgium (poster presentation)

23rd International Society for Environmental Epidemiology conference
Sep 13-16, 2011, Barcelona, Spain (poster presentations)

2nd Young Environmental Scientists Meeting
Feb 28 – March 2, 2011, Aachen, Germany (oral presentation)

SETAC Europe 3rd Special Science Symposium
Feb 2-3, 2011, Brussels, Belgium (poster presentation)

SETAC Europe 20th Annual Meeting
May 24-28, 2010, Seville, Spain (poster presentation)

BNS Annual Congress: Lipids in Nutrition
April 23, 2010, Brussels, Belgium (oral + poster presentation)

10th Flemish Youth Conference of Chemistry
March 1-2, 2010, Blankenberge, Belgium (poster presentation)

PVC Plasticisers Conference
Feb 10-11, 2010, Brussels, Belgium

2nd symposium 'Chemical safety of the food chain: Recent scientific developments'
Nov 24, 2009, Tervuren, Belgium